THE UNIVERSITY OF ALBERTA

RENAL REGULATION OF EXTRACELLULAR FLUID VOLUME

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

by

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EDMONTON, ALBERTA

1961

ABSTRACT

Renal responses to known expansion of blood volume, measured by a dye dilution method, have been studied in differentially hydrated, anesthetized dogs, using an "artificial blood" as the infusate. The latter consisted of washed red blood cells suspended in Ringer-Locke's solution containing 6% by weight of bovine albumin powder. This infusate was employed to eliminate histamine-like reactions due to plasma specificity known to follow homologous blood infusions in dogs.

Despite similar initial blood volumes and comparable increases of blood volume, infusion in a series of ten normally hydrated dogs resulted in larger diuretic and natriuretic responses than those seen in ten animals which had been thirsted for twenty-four hours prior to the experiment. A further group of ten dogs orally prehydrated with 50 ml/kg of normal saline 18 hours prior to the experiment also showed initial and post-infusion blood volumes comparable to those of the other series, but the magnitudes of diuresis and natriuresis exceeded markedly those of either of the other groups. These differences in response, which were significantly related to blood volume expansion in hydrated and normal but not in dehydrated animals, imply the operation of a volume regulatory mechanism for intravascular volume, which is modified appropriately by interstitial fluid volume. Since the mechanism of the renal response could not be satisfactorily explained on the basis of hemodynamic changes, the diuresis and natriuresis are believed to have been

due to a reflex influence on secretion of antidiuretic hormone and of a hypothetical natriuretic substance acting at the tubular level. An interesting but unexplained finding was an opposite change in effective renal plasma flow following infusion in dehydrated as compared with normal animals.

Comparison of renal function in ten prehydrated control animals with that of ten saline-loaded dogs which were infused with artficial blood while an equal quantity of circulating blood was removed, indicated that effects of infusate other than volume expansion could not explain the renal response following infusion. The minimal diureses which were observed in the exchanged group could be explained on the basis of dilution of circulating hormone.

ACKNOWLEDGEMENTS

I am deeply indebted to my advisor, Dr. J. W. Pearce, whose encouragement made this work possible and whose contribution to both the experimental work and the interpretation of results was invaluable.

Appreciation is extended to Mr. Jaro Mares for the preparation of figures.

I should also like to thank my wife, Clara, for her encouragement and understanding.

Personal and experimental support by means of a grant from the National Research Council of Canada to Dr. J. W. Pearce is gratefully acknowledged.

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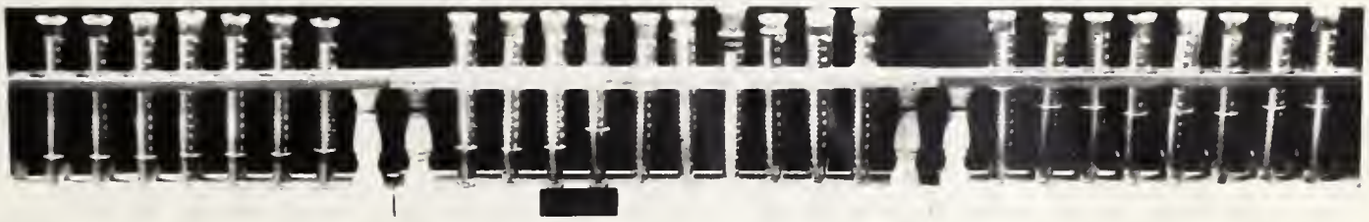

Urine samples taken at 10 min. intervals in an anesthetized dog infused during period indicated by black bar with an amount of blood expander equal to approximately 20% of circulating blood volume.

INTRODUCTION

In various studies of renal function in the dog, designed to establish a role of the kidney in the regulation of circulatory blood volume, infusions of different blood volume expanders have been shown to result in diuretic and natriuretic response (Zuidema et al, 1956; Pearce, 1959; Atkins and Pearce, 1959). To rule out effects of inusate other than volume expansion, studies were undertaken in this laboratory using homologous whole blood as a means of expanding the intravascular volume of dogs. The variable response obtained led to further studies using the Evans blue dye dilution method of plasma volume determination in an effort to relate blood volume changes to renal response. A consistent loss of infused plasma volume was found, although no overt signs of allergic reaction were noted. Bliss, Johns and Burgen (1959) and Remington and Baker (1959) demonstrated that homologous plasma infusion in dogs resulted in histamine-like reactions which were reduced or eliminated when autologous plasma was the infusate.

In view of these findings autologous blood infusions were given to a series of dogs. However, a loss of infused plasma similar to that observed in the series of homologous infusions was seen in these animals. Since precursors of polypeptides affecting capillary permeability could have been activated in the blood used for infusion

(Rocha e Silva, 1955; Lewis, 1958; Margolis, 1959), it was decided to use as the infusate an "artificial blood", consisting of washed red cells resuspended in Ringer-Locke's solution containing six per cent bovine albumin. Although a modification of the serial dye injection technique for determining plasma volume gave good agreement with expected volume increases, the renal response to infusion remained variable.

Since Ladd (1951) and Birchard and Strauss (1953) demonstrated that the state of hydration may be an important factor in determining renal response to intravascular volume expansion, the work reported in this thesis was undertaken in an effort to relate the state of hydration to the diuresis following blood volume expansion. In addition, direct renal effects of the infusate on renal function were to be determined by means of an isovolemic exchange of artificial blood. Finally, a control series of dogs was to be used to separate spontaneous variations in the different parameters measured from changes due to experimental maneuvers.

LITERATURE SURVEY

The cells of all higher animals are surrounded by an internal fluid which serves to maintain the cellular environment within the relatively narrow limits for normal cell function and thereby for life itself. Since active cells take up food substances from, and excrete metabolic waste products into this internal fluid, and since the intake of salts and water by the organism also tends to disturb the fluid balance, provision for maintenance of the constancy of the internal environment must exist. Starling in 1909 stated that the kidney must be considered the organ mainly responsible for the maintenance of volume and composition of the internal medium. At the present time the kidney is believed to be merely the effector organ, responding indirectly to changes in fluid volume and solute composition which are sensed by receptor systems located elsewhere in the body. Comprehensive reviews of blood volume regulation are available (Smith, 1957; Grossman, 1957; Pearce, 1961) and in the following sections some of the regulatory mechanisms of blood volume as the major controlling factor of extracellular fluid volume are outlined.

Plasma Osmotic Pressure

Verney in 1947 studied the effect of injections of hyperosmotic solutions into the common carotid artery of dogs and was able to demonstrate an inhibition of previously induced water diuresis. This

response was very much reduced by posterior hypophysectomy. Later work (1957) also demonstrated the ineffectiveness of adrenalectomy, vagotomy, and abdominal sympathectomy in abolishing the inhibitory response. The results obtained led to the conclusion that structures sensitive to changes in plasma osmotic pressure and supplied with blood from the common carotid artery influenced the secretion of antidiuretic hormone from the posterior pituitary gland. In an effort to locate the osmoreceptors Verney used histological and ablation studies in combination with studies involving changes of blood supply to various brain areas and concluded that the receptor site was in the anterior hypothalamic region of the brain.

The mechanism described is effective in maintaining the solute composition and indirectly the volume of extracellular fluid through variation of water output by the kidney. If, however, changes in fluid volume are unaccompanied by corresponding changes in plasma osmotic pressure, other homeostatic mechanisms must be active.

Blood Volume

a) Redistribution of Blood

Changes in posture have long been known to influence elimination of water and solutes by the kidney (White, Rosen, Fischer and Wood, 1926; Ni and Rehberg, 1931; Bazett, Thurlow, Crowell and Steward, 1924; Simpson, 1929; Brun, Knudson, and Raaschou, 1945). Epstein and co-

workers in 1951 confirmed earlier results that orthostasis caused a contraction of plasma volume, a decrease in renal clearances, and a fall in urinary water and sodium output. Infusions of isotonic albumin solution to return plasma volume to normal were without effect on the antidiuresis and therefore it was concluded that the distribution of blood was the regulating factor in urinary salt and water excretion changes due to orthostasis.

Brun and associates in 1946 investigated the mechanism of urine flow decrease during orthostasis and found the cause to be an increased secretion of anti-diuretic hormone. Confirmation of these findings was obtained by Judson and co-workers in 1950. In their experiments venous congestion of the limbs of patients suffering from diabetes insipidus did not result in a decrease of urine flow, although decreases in glomerular filtration rate, renal plasma flow, and urinary salt output were comparable to changes in normal subjects in whom an oliguria was observed following the same experimental procedure. The results obtained by these investigators indicate the existence of separate controlling mechanisms for urinary water and solute excretion, responding to redistribution of blood. The experiments of Pearce, Newman and Birmingham (1954) also point to an independent regulation of salt and water excretion and indicate antidiuretic hormone secretion as the factor controlling urine flow during orthostasis.

Strauss and his co-workers in 1951 reported that ingestion

of saline led to a prompt diuresis in recumbent subjects, whereas in upright subjects the saline load was eliminated over a much longer period of time. These investigators suggested that volume receptors in the thoracic region were stimulated by the shift in blood volume to the chest on lying down.

Drury, Henry and Goodman in 1947 had shown that positive pressure breathing in humans resulted in a mild antidiuresis, attributed by them to the stress involved in this maneuver. Since the results obtained could have been due to a shift in blood volume away from the thoracic area, Gauer, Henry, Sieker and Wendt (1954) investigated the effect of negative pressure breathing in the dog and noted that a mild water diuresis usually resulted. This study was repeated in humans by Sieker, Gauer and Henry in 1954 and by Hulet and Smith in 1959, who confirmed the earlier results.

In 1954 Henry, Gauer, and Reeves tried to localize the postulated thoracic volume receptors. Reasoning that the most distensible part of the circulation was the logical site for these receptors, the investigators engorged the pulmonary circulation of dogs by obstruction of pulmonary veins and distended the left atrium by means of an inserted balloon. Only the latter maneuver resulted in a large, prompt water diuresis. This reponse, as well as the diuresis following negative pressure breathing, was prevented or very much reduced by block of vagus nerves. The results obtained implicated stretch receptors

located in the atria as the sensing mechanism of blood volume increase. Vagal activity arising in atrial stretch receptors, which had been demonstrated by Paintal in 1953, was shown by Henry and Pearce (1956) to increase markedly with atrial balloon distension. It seems logical to conclude, therefore, that increased receptor discharge due to an increase in atrial filling volume results in a reflex inhibition of antidiuretic hormone and a consequent increase in water output. This conclusion is supported by the findings of Baisset (1958) and his coworkers, who measured antidiuretic hormone levels in venous blood of dogs during and following atrial balloon distension. Verification of the results of Henry, Gauer, and Reeves was obtained and in addition biological assay demonstrated a consistent decrease of antidiuretic material in the blood of the experimental animals during atrial distension. However, recent evidence by Ledsome, Linden, and O'Connor (1960) contradicts these findings, since administration of pitressin was without effect on the diuresis induced by atrial stretch.

Even if an inhibition of antidiuretic hormone is accepted as the cause of the increased urine flow, another mechanism must be postulated for the observed decrease in urinary solutes during orthostasis and for the natriuresis accompanying shifts of blood volume from peripheral to central areas.

b) Intravascular volume expansion

As early as 1900 Thompson reported diuresis in anesthetized dogs following infusion of small quantities of normal saline. These diuretic responses were preceded by increases in urinary chloride and urea. Metcalf in 1944 infused serum and plasma into anesthetized dogs and reported an increase in urine flow within one hour after infusion. Blood volume expansion in human patients by means of oral saline and water loads was studied by Blomhert and his associates in 1951. These investigators found that while ingestion of water led, as expected, to a water diuresis, the administration of saline resulted in a biphasic renal response in which natriuresis was preceded by diuresis. Strauss and his colleagues (1951) demonstrated that infusion of isotonic saline resulted in diuresis only if the subjects were in the recumbent position. Papper et al. (1956) also observed a natriuresis following isotonic saline infusion in recumbent subjects.

In order to eliminate the variable of transudation of fluid into the interstitial fluid space which is characteristic of saline infusions, Welt and Orloff in 1951 studied the effect of isooncotic albumin infusion in humans and demonstrated that the resulting marked diuretic response could be blocked by administration of pitressin. Therefore these workers suggested that plasma volume expansion led to inhibition of antidiuretic hormone secretion and thereby induced diuresis.

Zuidema and co-workers (1956) reported a diuresis following

infusion of normal saline, iso-oncotic albumin solution, plasma, and whole blood in dogs. The response was attributed to an increased atrial stretch receptor discharge leading to antidiuretic hormone inhibition as postulated by Henry, Gauer and Reeves. Since no information on changes of glomerular filtration rate and urinary composition was obtained, the validity of this conclusion may be questioned. The diuretic and natriuretic response to iso-oncotic bovine albumin infusions in the dog was shown to be independent of the integrity of vagal and carotid sinus nerves (Pearce, 1959), suggesting a different mechanism of blood volume regulation from that proposed to respond to negative pressure breathing. Additional evidence implicating a second regulatory mechanism was obtained by Atkins and Pearce in 1959. Comparing the effects of vagotomy on the diuretic and natriuretic response of normal and adrenalectomized dogs to infusions of canine plasma, these workers found that postvagotomy response to infusion was not abolished in either series, although a reduction of magnitude from prevagotomy response was observed. Changes in glomerular filtration rate were variable, suggesting a tubular mechanism. However, filtration changes too slight to be detected by present methods can not be excluded as a contributing factor to diuresis and natriuresis following intravascular volume expansion.

Regulation of blood volume by means of variation in renal excretion of sodium also would be effective in the maintenance of the

constancy of internal environment.

c) Sodium excretion by the kidney

(i) Aldosterone

Since Simpson and co-workers in 1953 isolated the powerful salt-retaining hormone aldosterone from preparations of adrenal cortex, research into its role in blood volume regulation has been progressing. Ganong and Mulrow (1958) studied the effect of injection of aldosterone into the aorta of dogs and reported a decreased sodium and increased potassium excretion unaccompanied by any consistent change in filtration rate. A consistent delay of five to thirty minutes between injection of aldosterone and onset of action was observed. Following administration of aldosterone to unanesthetized dogs in a state of sodium diuresis Barger, Berlin and Tulenko (1958) were able to demonstrate a moderate decrease in urinary sodium excretion which persisted for several hours after administration of the hormone was stopped. These findings may indicate that the role of aldosterone consists of long-term rather than immediate regulation of sodium output. The fact that the diuresis of atrial distension is not associated with a natriuresis (Gauer et al., 1954; Hulet and Smith, 1959; Ledsome et al., 1960), despite a reduction in aldosterone blood levels following sustained mechanical stretching of the atria (Anderson, McCally, and Farrell, 1959), also suggests that this hormone does not initiate immediate

changes in urinary sodium excretion.

Bartter and co-workers in 1956 observed that dietary sodium restriction or dehydration in humans resulted in an increased urinary aldosterone level and decreased sodium excretion. These effects could be reversed by sodium and water administration. They concluded that an expansion of extra-cellular fluid volume initiated the reduction of aldosterone, thus increasing sodium output.

Mills, Casper and Bartter (1958) described a mechanism of control of secretion of aldosterone. Constriction of thoracic inferior vena cava in anesthetized dogs, which would decrease the filling volume of the heart, consistently increased the secretion of aldosterone within one hour of application of the constriction. Release uniformly resulted in a fall of secretion. The effect of constriction could be nullified by rapid infusion of blood cephalad to the constriction, suggesting that blood volume did indeed play a part in aldosterone regulation. The role of the buffer nerves in this regulation was investigated also. It was found that bilateral vagotomy in the dog did not prevent the rise in aldosterone secretion seen on caval constriction, but that it did inhibit the return to normal which usually followed release of constriction. Constriction of the common carotid arteries in the intact animal resulted in an enhanced secretion of aldosterone. This response was abolished by denervation of the carotid arteries; however, careful denervation of the carotid sinus alone did not seem

to interfere with the mechanism of increase of aldosterone secretion. Subsequent investigation (Bartter, Mills and Gann, 1959) showed that denervation of the thyrocarotid junction was effective in inhibiting the above response. In an experiment in the dog the aldosteronism produced by chronic constriction of the thoracic vena cava was reversed by denervation of the thyrocarotid junctions. A possible effector mechanism for aldosterone regulation may be suggested from work done by Farrell. A tropic factor specific for the zona glomerulosa of the suprarenal gland was found in saline extracts of beef diencephalon (1959a) and the active principle was finally obtained from pineal extracts (1959b). An inhibitory factor for aldosterone secretion could also be isolated from the pineal gland. Interaction of tropic factor (Glomerulotropin), inhibitory factor (antiglomerulotropin), and adrenocorticotropic hormone is suggested for regulation of aldosterone secretion (Farrell, 1960).

The above investigations indicate a mechanism of control of aldosterone secretion which is sensitive to changes of intravascular volume: Decrease in intravascular volume with an associated decrease in atrial filling volume and arterial pulse pressure inhibit receptor discharge from the atria and thyrocarotid junctions, which results in a reflex secretion of glomerulotropin from the area of the pineal gland. Glomerulotropin then stimulates the glomerular zone of the adrenal cortex to secrete aldosterone, which in turn causes retention

of sodium by the kidney. Release of aldosterone may be inhibited by increased atrial distention, possibly by means of liberation of inhibitory factor and inhibition of release of corticotropin. Hypophyseal adrenocorticotropic hormone may also contribute to aldosterone regulation.

Undoubtedly the aldosterone mechanism is not the only one for regulation of sodium excretion. Atkins and Pearce demonstrated a natriuresis in adrenalectomized dogs, and studies of sodium excretion in patients suffering from Addison's disease also point to regulation of sodium balance independent of the aldosterone mechanism (Rosenbaum, Papper and Ashley, 1955; Taymor and Friedberg, 1957).

ii) Hemodynamic factors

Several reviewers (Selkurt, 1954; Smith, 1957; Grossman, 1957; Welt, 1960; Pearce, 1961) have pointed out that changes in filtration rate which fall within the experimental error of present methods of measurement may increase the tubular sodium load and in the absence of reabsorption changes lead to a rise in excretion of sodium. It is well known that saline load in dogs results in increased glomerular filtration rate (Ladd and Raisz, 1949; Selkurt and Post, 1950). While filtration changes in man are less consistent,corresponding to a less efficient elimination of saline load (Smith, 1957), glomerular filtration changes can not be ruled out in the regulation of sodium and water balance.

A second factor which may influence excretion of solute and water consists of changes in renal medullary blood flow. By means of photoelectric measurements of dye-passage times in renal medulla Thurau, Deetjeu, and Kramer (1960) were able to show increases in medullary blood flow with increasing water and osmotic diuresis, returning to the original values with decreasing urine flows. These investigators also demonstrated that, in contrast to renal cortex, no autoregulation of blood flow took place in the medulla, relatively large flow changes being observed with changes in perfusion pressure. In view of these results Ochwadt (1959) speculates that changes in volume and concentration of urine may be partially explained on a hemodynamic basis by assuming changes in the medullary countercurrent concentration gradient due to variations in medullary blood flow.

(iii) Natriuretic hormone

Recent work suggests yet another possible mechanism of sodium control; Keller in 1959 produced hypothalamic lesions in the area of the paraventricular nuclei in rats and observed a consistent rise in urinary sodium excretion which was not eliminated by renal denervation, hypophysectomy, chronic bilateral adrenalectomy or by reduction of extracellular fluid volume. Since renal nervous pathways and aldosterone secretion seemed to be ruled out, the author suggested that a natriuretic hormonal influence could explain the results obtained. A natriuretic hormone was also proposed by Wise and Ganong (1960) to

explain the rise in urinary sodium excretion occurring with electrical stimulation of points at the pontomedullary junction of the brainstem of anesthetized dogs. The response was shown to be independent of renal innervation and no consistent filtration changes were observed.

Evidence for a direct natriuretic hormone is still speculative and further investigation is necessary.

Interstitial Fluid Volume

In the homeostatic maintenance of body fluids the regulation of intravascular volume can be considered as of primary importance, due to the carrier function of blood. All food materials are transported to sites of utilization, and similarly, waste products of cell metabolism enter the bloodstream and are carried to specific sites of elimination. It seems reasonable to expect the operation of a mechanism maintaining the volume of the intravascular compartment at the expense of other fluid spaces during periods of reduced or increased fluid intake.

Such a mechanism is provided by the wellknown Starling-Landis hypothesis. Reduction of plasma water during periods of dehydration leads to a relative rise in plasma oncotic pressure, resulting in shifts of fluid from the interstitial and ultimately from the intracellular space into the intravascular compartment. The resulting cellular dehydration leads to the sensation of thirst and an increased

water intake (Gilman, 1937). Limited hemorrhage also results in shifts of tissue fluid into the circulatory system, due to a fall in venous pressure, so that total blood volume returns rapidly to normal levels.

Conversely, ingestion of fluid, which leads to a relative decrease in plasma oncotic pressure, or infusions, which raise the hydrostatic pressure affecting capillary filtration, result in transudation of fluid into the interstitial compartment. Recent work by Walker, Ross and Hammond (1960) suggests a third possibility for effecting transcapillary movement of water. A relation between plasma volume and transcapillary exchange rate of plasma albumin was observed in human hospital patients. Since the same relationship was found to exist between plasma volume and globulin exchange rate, these authors explain the results on the basis of apparent capillary permeability changes, which serve to maintain plasma volume within narrow limits at the expense of the interstitial fluid space. The work of Mellander (1960) indicates that sympathetic nervous tone may also play a part in regulating the exchange of fluid between the intravascular and interstitial compartments.

These mechanisms - and the ones discussed under plasma osmotic pressure and blood volume - thus provide for adequate regulation of the intravascular component of body water. This regulation alone could not maintain homeostasis, since fluid intake after prolonged dehydration would activate vascular volume receptors leading to excretion of

fluid needed by the body. Therefore, from a teleological point of view, receptors transmitting information about interstitial fluid volume are a necessity if the cellular environment is to be kept constant. The relatively large volume changes which occur in the interstitial space provide an excellent stimulus for the proposed receptors.

a) Reduction in interstitial fluid volume

Welt and Orloff (1949) realized the possibility of such a regulatory mechanism. Studying the effects of infusions of concentrated albumin solutions in normal subjects, these investigators observed a fall in renal excretion of sodium and water despite a markedly elevated plasma volume. Since this elevation occurred at the expense of the interstitial fluid space, the authors considered the possibility that "a decrease in interstitial fluid volume in normals initiates a mechanism that increases the re-absorption of sodium and water." A similar investigation by Goodyer, Peterson and Relman (1949) confirmed the results obtained by Welt and Orloff. An infusion of a solution containing seventy-five grams of human albumin during water and mannitol diuresis in man resulted in a twenty per cent increase of plasma volume. However, the urine flow and urinary sodium and chloride excretion decreased markedly. These decreases, and those observed by Welt and Orloff, were unaccompanied by changes in glomerular filtration rate, indicating the operation of a tubular mechanism.

Petersdorf and Welt (1953), in an effort to elucidate the mechanism of renal response to hyperoncotic albumin solution, administered twenty-five per cent albumin to normal human subjects during maximal water diuresis and to patients suffering from diabetes insipidus. In both groups a rise in plasma volume was accompanied by a fall in renal water, sodium, potassium, and chloride excretion. The similarity in response between normal and diabetic subjects suggests a mechanism which is independent of the secretion of antidiuretic hormone. From the data obtained Petersdorf and Welt inferred that the increased sodium reabsorption caused a passive reabsorption of water. Since the reduction in sodium output was also observed in an Addisonian patient (Welt and Orloff, 1949), the antinatriuretic effect seems to be independent of the aldosterone mechanism.

The above conclusions remain to be reconciled with the findings of Orloff and Blake (1951) in conscious dogs. Hydrated animals uniformly responded to hyperoncotic albumin with a substantial diuresis accompanied by a relatively smaller rise in sodium and potassium excretion. The diuresis was inhibited by administration of pitressin, indicating a tubular mechanism for the increased water elimination. Hydropenic animals showed a smaller and less consistent diuretic response, and a tendency toward sodium conservation was observed. These results may indicate a species difference between man and dog in the

b) Increase in interstitial fluid volume

Since reduction in interstitial fluid space leads to conservation of fluid and electrolytes, reversing this procedure might be expected to potentiate diuresis and natriuresis. Ladd (1951a, 1951b, 1952) in a series of experiments compared the renal responses to saline infusion in slightly dehydrated and prehydrated subjects. The former group responded to infusion with a moderate diuresis. Subjects which had been prehydrated with two liters of water eight to thirteen hours prior to administration of saline responded with a large diuresis which could be inhibited by injection of pitressin. In contrast to the first group hypotonic urine was eliminated. Excretion of sodium was also enhanced following prehydration, the natriuresis remaining unaffected by pitressin. Filtration rate was seen to increase following infusion, but the increase could only partially explain the rise in sodium output, which therefore must be attributed in part to decreased tubular reabsorption.

While these results seem to substantiate the theory of interstitial fluid regulation, a discrepancy remains apparent: It was found that prehydration at less than eight hours prior to saline administration was less effective in increasing urine flow than prehydration from eight to thirteen hours prior to infusion. The solute urine to plasma ratio rose, however, which could indicate a comparable natriuretic influence due to interstitial volume expansion. A delayed

change of responsiveness of the neurohypophyseal antidiuretic hormone secretory system is postulated by Ladd to explain the changes in diuresis with time of prehydration. A delayed, indirect effect of interstitial volume expansion could conceivably affect this responsiveness.

Birchard and Strauss in 1953 performed a similar experiment except that oral saline loading the previous day was followed by ingestion of saline. While the ingestion of saline normally did not cause diuresis in erect subjects, prehydration resulted in good diuresis and production of hypotonic urine. The increase in water output was unaccompanied by filtration changes. These results led to the inference that the supraoptical-hypophyseal system responded to changes in volume or distribution of extracellular fluid. Smith (1957), in reviewing these results, disagreed with the conclusion, since a large infusion of isotonic saline in sitting subjects, which undoubtedly increases extracellular volume, does not result in diuresis. He suggested a subliminal inhibition of the antidiuretic system due to preloading with saline as the causative factor. A satisfactory explanation awaits further investigation.

Balint and Pethes in 1959 extended the studies of Ladd to the unanesthetized dog by comparing the results of large, rapid isotonic saline infusions in dehydrated and water-prehydrated animals. No difference in natriuresis was seen between the two groups, but a transient hypotonic phase in the larger diuresis of prehydrated dogs was observed.

The equal magnitude of rise of glomerular filtration rate in both series may explain the natriuresis, and the finding that prehydration did not affect sodium excretion remains to be reconciled with the proposed regulatory mechanism of interstitial fluid volume.

It seems obvious from the above investigations that the simple theory of interstitial fluid regulation proposed at the beginning of this section is inadequate. An interaction of interstitial with intravascular volume regulation and other, as yet unknown, factors must be postulated to explain the experimental results obtained by various investigators.

In summary, regulation of osmotic pressure of body fluids by means of a mechanism consisting of osmoreceptors which initiate reflex secretion and possibly inhibition of antidiuretic hormone provides an indirect control of volume changes, if these are accompanied by appropriate osmotic pressure changes. In addition, a complex system of intravascular volume regulation exists, the reflex portion of which depends on the activity of receptors located in both the high and low pressure circulation. Atrial stretch receptors may control a reflex secretion of antidiuretic hormone and possibly influence secretion of a hormone tropic for aldosterone. Receptors located along the carotid artery regulate the secretion of aldosterone by varying the output of tropic hormone. Additional control of sodium excretion exists and may be mediated through renal hemodynamic changes or through the release

of a natriuretic hormone. Some evidence for participation of a parameter of interstitial fluid space in volume regulation exists, but the mechanism of this regulation is unknown.

METHODS

Experimental Plan

Investigations on the anesthetized dog were carried out to arrive at a quantitative relationship between intravascular volume expansion and renal response (consisting of changes in glomerular filtration and renal plasma flow and variation in water and solute excretion). In an attempt to standardize the conditions of the experiment, anesthetic level was maintained as uniformly as possible, body temperature was measured and controlled, and changes in plasma osmotic pressure were determined. In addition a consistent time schedule was adhered to throughout the experiment.

Preliminary investigations using homologous and autologous whole blood infusions resulted in variable and unpredictable volume expansion due presumably to histamine-like reactions. Therefore a nonreactive material having many of the physical properties of circulating blood was used as the infusate. Since the state of tissue hydration may modify the renal response to intravascular volume expansion, as outlined in the literature review, fluid intake was standardized, and the effect of controlled changes in this variable was determined.

Five series of ten experiments each were performed, using as the infusate an artificial blood, the make-up of which is described

below. Effects of infusion into normal dogs (Series I), dehydrated dogs (Series II), and prehydrated animals (Series III) were compared. In addition to control experiments in prehydrated dogs (Series V), possible direct pharmacological actions of the infusate and effects of dilution of circulating hormones were evaluated in a group of prehydrated dogs receiving an infusion of artificial blood coupled with simultaneous removal of an equal amount of the animal's own blood (Series IV).

The sequence of experiments in Series III to V was randomized statistically and an analysis of variance of the split-plot type was carried out. A similar analysis was used to compare results of Series I to III.

Preparation of the Animal

Mongrel dogs of both sexes weighing from 8 to 15 kg. were used. The dogs of Series I were allowed water ad libitum until the experiment, the dogs of Series II were thirsted for twenty-four hours prior to the experiment, and those of Series III to V were prehydrated with 50 ml. per kg. body weight of normal saline given by stomach tube eighteen hours before the start of the experiment.

The animals were anesthetized by intravenous injection of sodium pentobarbital (35 mg./kg.) and a depth of anesthesia just sufficient to prevent spontaneous movement was maintained by 15 mg. doses

of anesthetic at ten to twenty minute intervals. Arterial blood pressure was measured by means of a mercury manometer connected to a cannula inserted in the left femoral artery and right atrial pressure changes were determined with a saline manometer connected to a catheter inserted into the atrium via the external jugular vein. The manometer was adjusted to read zero with the recording column level with the upper end of the sternum. The right femoral vein and both forearm veins were exposed for subsequent injection of Evans-blue dye. Both ureters were cannulated with fine polyethylene tubing through a suprapublic midline incision. Upon completion of the operative procedures a priming dose of creatinine and PAH (p-aminohippurate) was administered through a polyethylene catheter tied into the left femoral vein. This was followed by a constant slow infusion of an isosmotic solution of creatinine and PAH. An equilibration period of one hour was allowed to elapse before any measurements were made.

Experimental Schedule

The time schedule followed in the experiments is shown in Fig. 1. Measurements of the parameters indicated at the beginning of the schedule were taken throughout the experiment, while other variables were measured at the specified times. Three consecutive determinations of G.F.R. (Glomerular filtration rate) and E.R.P.F. (effective renal plasma flow) were made during the second half of

EXPERIMENTAL SCHEDULE

Time (min.)	Readings		
10	U.V., $U_{Na^+}V.$, $U_{K^+}V.$, $U_{Osm}V.$, B.P., R.A.P.		
20			
30			
40			
50			
60	Control period		
70		G.F.R., E.R.P.F.,	Blood sample no.1 at 64 min. / 1 ml. dye at 66 min.
80		P.V., B.V.	Blood sample no.2 at 76 min.
90			Blood sample no.3 at 85 min.
100			Infusion (Exchange; Control)
110			
120		P.V., B.V.	Blood sample no.4 at 115 min.
130			
140	Infusion period		
150		G.F.R., E.R.P.F.,	Blood sample no.5 at 144 min. / 1/2 ml. dye at 146 min.
160		P.V., B.V.,	Blood sample no.6 at 156 min.
170			Blood sample no.7 at 165 min.
180			
190			
200		P.V., B.V.	Blood sample no.8 at 195 min.
210			
220	Post-infusion period		
230		G.F.R., E.R.P.F.,	Blood sample no.9 at 225 min. / Blood sample no 10 at 234 min.
240		P.V., B.V.	1/2 ml. dye at 236 min. / Blood sample no.11 at 246 min.
250			

Figure 1: Experimental schedule for

Series I to V

Abbreviations:

U.V.	= urine volume in ml/10 min.
$U_{Na}+V$	= urinary sodium excretion in μEq./10 min.
$U_{k}+V$	= urinary potassium excretion in μEq./10 min.
$U_{Osm}V$	= urinary solute excretion in μOsm./10 min.
B.P.	= arterial blood pressure in mm Hg.
R.A.P.	= right atrial blood pressure in cmsaline.
P.V.	= plasma volume in ml.
B.P.	= blood volume in ml.
G.F.R.	= glomerular filtration rate in ml/kg/min.
E.R.P.F.	= effective renal plasma flow in ml/kg/min.

each of the main periods. Blood samples used for estimation of plasma and blood volumes and for assessment of plasma creatinine and PAH levels were withdrawn through a catheter tied into the right femoral vein following injection of the initial dose of dye.

Preparation, Infusion, and Exchange of Blood Volume Expander

Arterial blood*, withdrawn into sterile containers from donor or experimental animals, was stored at 4° centigrade in "ACD" solution until use (up to one week). The blood was then centrifuged for fifteen minutes at 2000 r.p.m. and the plasma was discarded. The red blood cells were resuspended in 0.9% saline and the supernatant was drawn off after centrifugation. This procedure was repeated twice. The washed red blood cells were diluted with an equal volume of mammalian Ringer-Locke's solution containing 6% of bovine albumin powder (Fraction V; Armour Pharmaceutical Co.). The volume of infusate prepared was equal to twenty-five per cent of the estimated blood volume of the recipient animal, assuming a blood volume of 85 ml./kg. body weight.

The artificial blood was infused at a rate of approximately 1.0 ml./kg./min. through the right femoral vein catheter by means of a gravity drip.

An isovolemic exchange of artificial blood for blood from the experimental animal was obtained by using the apparatus sketched in

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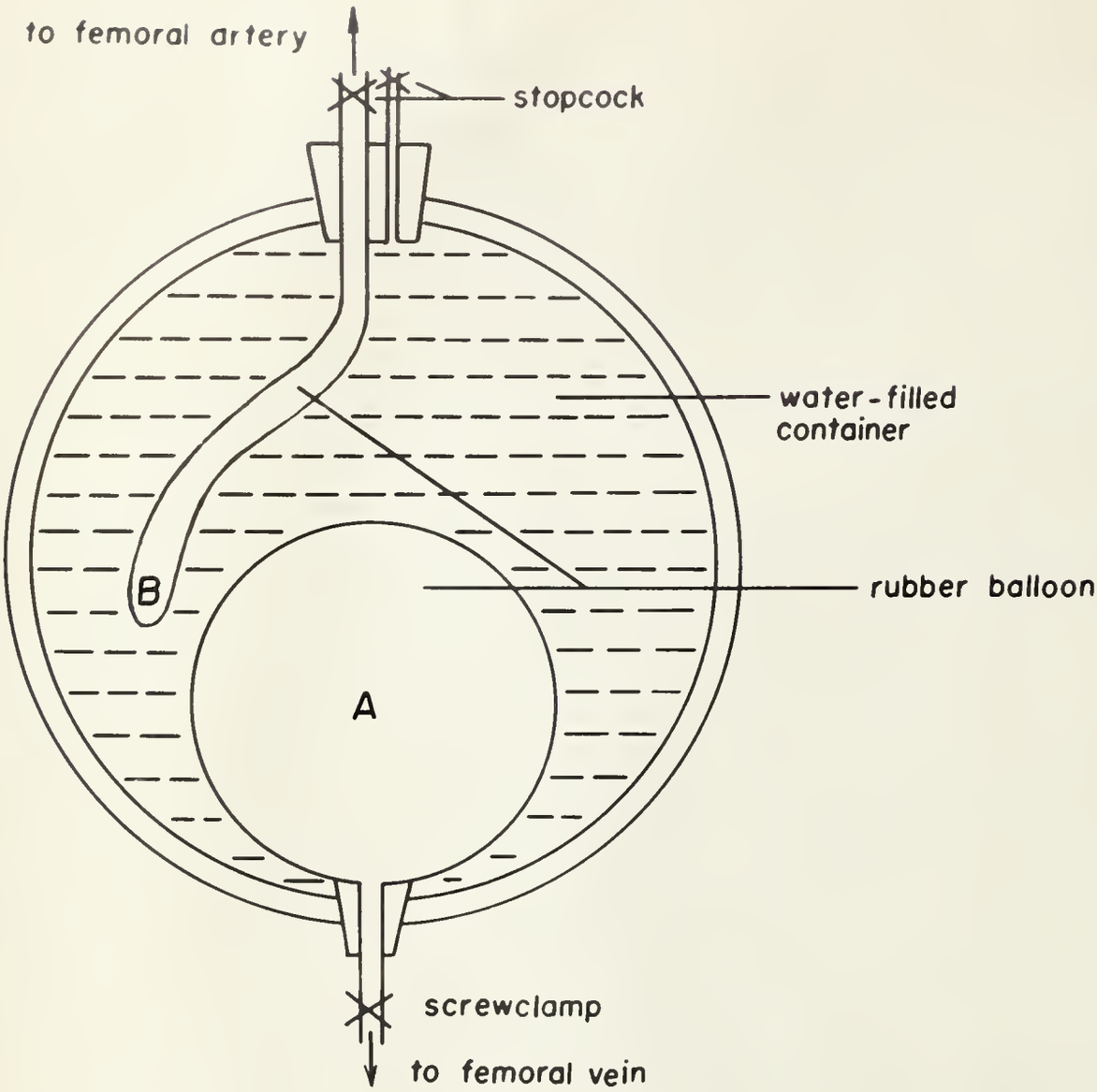
*(homologous blood only was used)

EXCHANGE APPARATUS



DYE CURVE

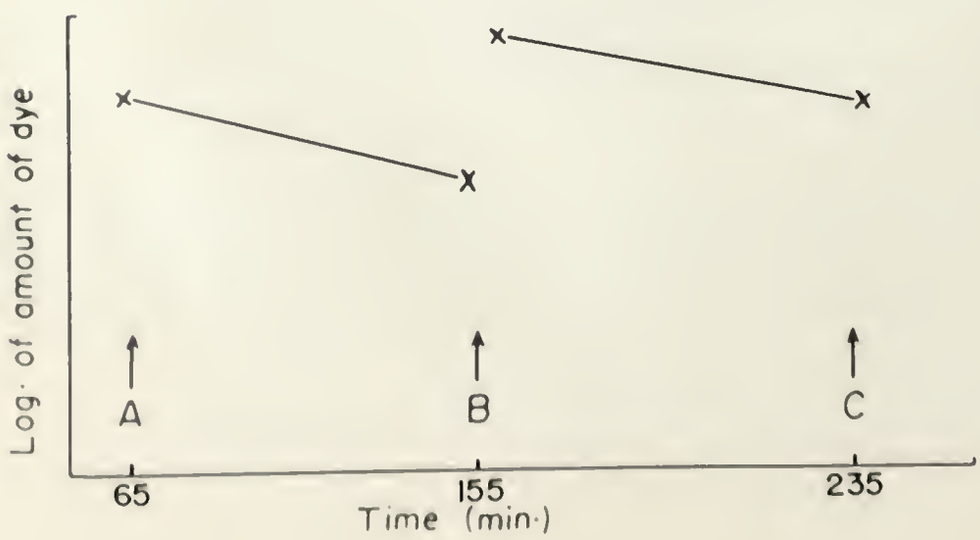


Figure 2a: Apparatus used in the exchange of artficial blood for the blood of the recipient animal. As arterial blood is allowed to enter balloon B an equal volume of artificial blood is expressed intravenously.

Figure 2b: A schematic plot of log. of plasma content of Evans-blue dye against time. Injection of dye takes place at points A, B, and C, as outlined in the experimental schedule. Sample calculations of plasma volume are given below:

Point A - 5×10^{-3} gm of dye injected.
- P.V. = amount injected/concn. of 10 min. sample.

Point B - dye sampling and subsequent injection of 2.5×10^{-3} gm of dye.
- P.V. $\neq$ new amount injected/difference in concn. before and 10 min. after injection.
- Amount before injection = P.V. x concn. before injection.
- Amount after injection = amount before injection plus 2.5×10^{-3} gm.

Point C - repeat procedures under B.

A linear decline of log. of dye content with time is assumed in joining any two points.

An exact measurement of plasma volume can be made at selected times during the experiment by obtaining a sample of dye concentration at these times and determining the amount of dye in the circulation from the graph.

Fig. 2a. Two rubber balloons, A. and B., were inserted into a water-filled glass container as shown. Balloon A was filled with artificial blood and connected to the femoral vein while the empty balloon B was connected to an arterial catheter. By controlling the rate of outflow of artificial blood the arterial inflow was regulated, and an isovolemic exchange resulted.

Plasma and Blood Volume Determinations

Measurement of plasma volume by means of the dye dilution technique depends on the well-known principle that volume equals amount divided by concentration. The blue diazo dye T-1824 (Evans blue), which was first used by Evans and co-workers in 1920 to determine plasma volume, has been shown by Rawson (1943) to be firmly and selectively bound to the albumin fraction of plasma. Gregerson and Rawson in 1943 demonstrated a straight-line relationship between time and the logarithm of loss of dye from the circulation. Mixing of dye in the circulation under a variety of conditions was shown to be complete within ten minutes of injection (Noble and Gregerson, 1946). These investigators also found no significant difference between plasma volume calculated using a ten-minute concentration sample and plasma volume determined from extrapolation to zero time of a dye decay curve.

Using the following equation an estimation of blood volume can be made if plasma volume and the ratio of cells to plasma are known:

$$BV = \frac{PV}{(100-Hc't)} \times 100$$

BV = blood volume; PV = plasma volume; Hc't = body hematocrit. The hematocrit obtained from a blood sample must be corrected for plasma trapped in the column of packed red cells and a correction factor of 0.96 is suggested by Gregerson and Rawson (1959).

Since the concentration of red cells is not the same in all parts of the circulatory system, central venous hemotocrit does not necessarily represent the true overall percentage, and a second correction factor, the F_{cells} value, is derived from the ratio of overall hemotocrit to venous hemotocrit. In dogs anesthetized with Nembutal (sodium pentobarbital) the relaxed spleen contains large amounts of red cells at high concentration. Under these conditions the F_{cells} value was found to be 1.1 (Gregerson, 1953).

The modified equation

$$BV = \frac{P.V. \times 100}{(100-Hct \times 0.96 \times 1.1)}$$

was used in the investigations reported here to obtain estimates of total blood volume.

a) Standard dye curve

Two ml. of a 0.5% solution of T-1824 (Warner-Chilcott Laboratories) were measured with the syringe described below and diluted to

100 ml. with 0.9% saline. Two-ml. aliquots of this dye solution were then diluted with various volumes of saline so that eighteen dilutions of dye, ranging in concentration from $2x10^{-6}$ gm./ml. to $40x10^{-6}$ gm./ml. were obtained. One ml. of each of the final dilutions was added to 2 ml. of dog plasma and optical density was read against a saline-diluted plasma blank. A Fisher Electrophotometer with a filter allowing maximum transmission at 620 mu was used. A curve relating optical density to dye concentration was plotted and used to determine the concentration of dye in experimental plasma samples.

b) Injection of dye and sampling

A 1 cc. tuberculin syringe was marked on barrel and plunger to deliver 1 cc. and $\frac{1}{2}$ cc. volumes. To reduce dead space present in a needle a short length of twenty-gauge steel tubing was fastened into the front aperture of the syringe. Each amount of dye was injected into an exposed, previously unused vein at the times indicated in Fig. 1. Samples of blood were withdrawn at the appropriate times into clean, dry glass syringes and centrifuged immediately for ten minutes. Plasma (2 ml.) was removed by means of a pipette and diluted with 1 ml. of saline. Since no deterioration of dye under these experimental conditions had been found over a period of six hours, the concentration of all samples was determined upon completion of the experiment.

c) Calculations

Since blood volume changes in the experiments reported here disturbed the straight-line relationship between log. of dye concentration and time, a modification of the dye dilution method for measuring plasma volume was introduced. This modification is illustrated in Fig. 2b. The dye content of plasma before and after injection of fixed amounts of dye was calculated as shown and straight lines relating log. of amount of dye to time were drawn. An exact measurement of circulating plasma volume could be obtained at different times during the course of the experiment, using a dye concentration sample and determining the amount of dye from the graph. The validity of this method of determining plasma volume is borne out by the relative constancy of blood volume measurements in a series of control animals.

Renal Clearances

A substance in the blood which, on passing through the kidney, is filtered by the glomeruli and is neither excreted nor absorbed by the tubules may be used to measure the rate of glomerular filtration. If the concentration of the substance in plasma and urine is known, and if the volume of urine produced in a certain length of time is measured, the volume of plasma filtered during this period may be calculated as follows:

$$G.F.R. = \frac{UV}{P} \quad (= \text{Clearance of the substance})$$

where U = concentration in the urine, V = volume of urine produced per unit time, and P = concentration in plasma.

A substance in the plasma which is both filtered by the glomeruli and totally excreted by the tubules can be used to determine the total flow of plasma through functional renal tissue by applying a similar method to the one described above. Substances which satisfy these criteria in the dog are creatinine and p - aminohippurate (PAH) for G.F.R. and E.R.P.F. respectively.

a) Maintenance of plasma creatinine and PAH levels

A priming dose of creatinine and PAH dissolved in a minimum of distilled water was administered after completion of the operative procedure. The amounts of chemicals were determined from the equation:

$$\text{Priming Dose (gm)} = \text{dog wt (kg)} \times \frac{\text{vol. of distrib.}}{100} \times \text{intend. plasma conc'n (mg \%)}$$

The volume of distribution was found to be 50% of body weight for creatinine and 30% for PAH (Greenberg et al., 1952). Recommended plasma levels are 7 to 15 mg. % and 1 to 2 mg. % for creatinine and PAH respectively (Homer Smith, 1951). Following injection of the priming dose a sustaining intravenous infusion maintained plasma levels near the desired values by means of a constant pressure drip method. The con-

stant infusion was calculated as follows:

$$\text{Conc'n. (gm/ml)} = \frac{\text{plasma concn. (mg. \%)} \times \text{dog wt. (kg.)} \times \text{estimated clearance (ml./kg./min.)}}{\text{rate of infusion (ml./min.)} \times 1000}$$

Estimated clearance values for creatinine (4.3 ml./kg./min.) and for PAH (13.5 ml./kg./min.) were obtained from investigators by Smith (1951).

b) Standard curves and measurement of concentrations

Concentrations were determined by modifications of the Folin-Wu alkaline picrate method (Bonsnesc and Taussky, 1945) and the method of Smith and co-workers (1945) for creatinine and PAH respectively. A protein precipitating solution was prepared by adding 100 ml. of 10% sodium tungstate solution to 1500 ml. of water containing 100 ml. of 2/3 N sulfuric acid. When this solution was used immediately after preparation to precipitate plasma proteins, an interfering level of cloudiness developed in the filtrate on adding the color reagent for PAH. The cloudiness was found to decline to a low, steady value within thirty days after preparation of the precipitant and therefore at least one month was allowed to elapse before the solution was used.*

A standard plasma solution was prepared by dissolving 0.080 gm. of creatinine and 0.020 gm. of PAH in one liter of aged protein precipitant. Aliquots of 0.5 to 5 ml. of standard solution (with successive increments of 0.5 ml) were diluted to 17 ml. with protein precipitant

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*Any residual interfering effect is accounted for by using a plasma blank, which would also eliminate differences in reactivity of precipitant with different plasmas.

to give nine dilutions ranging in concentration from 2 to 20 mg. % of creatinine, and from 0.5 to 5 mg. % of PAH. Two ml. of normal canine plasma diluted with 1 ml. of saline were added to each dilution and mixed thoroughly. The mixture was allowed to stand for five minutes before being filtered.

(i) Standard plasma creatinine: Duplicate three ml. aliquots of filtrate in five-ml. Fisher Electrophotometer tubes were mixed with 1 ml. of 0.04 M picric acid and 1 ml. of $\frac{3}{4}$ N sodium hydroxide. The orange color due to a tautomer of creatinine picrate was allowed to develop for ten minutes and optical density was read against a plasma blank in a Fisher Electrophotometer, using a 540 mu filter. A standard curve relating optical density to concentration in mg. % was plotted from the mean readings of duplicates.

(ii) Standard plasma PAH: To two-ml. duplicate aliquots in five-ml. photometer tubes 0.4 ml. of 1.2 N hydrochloric acid and 0.2 ml. of 0.1% sodium nitrite were added and the solution was mixed thoroughly. After five minutes 0.2 ml. of 0.5% ammonium sulfamate was added and allowed to stand for three minutes following mixing. Coupling reagent (N - (1-naphthyl) ethylene diamine dihydrochloride, 0.2 ml.) was then added and the sample was shaken immediately. Ten minutes were allowed for development of the violet color and optical density was read against a plasma blank and plotted as before.

Fresh sodium nitrite was prepared every week and was stored in

the cold, together with ammonium sulfamate and the coupling reagent (0.1%). The coupling reagent was kept in a dark bottle to prevent breakdown due to light.

(iii) Measurement of plasma concentrations: Samples of diluted plasma, which had been used for the Evans-blue dye determination, were mixed with 17 ml. of protein precipitant, filtered after the appropriate length of time, and the concentration of creatinine and PAH was determined, using the methods outlined above.

(iv) Standard urine creatinine and PAH: A urine standard was prepared by dissolving 1.250 gm. of creatinine and 0.750 gm. of PAH in one liter of water. Different volumes of this standard were diluted to 100 ml. in volumetric flasks containing one ml. each of mixed normal dog urine.

Thirteen dilutions of creatinine, ranging in concentration from 250 to 3875 mg. %* were further diluted by a factor of one to ten and treated in the same manner as plasma filtrate. Optical density was read against a treated water blank and a diluted sample of mixed normal dog urine was taken to equal zero concentration, thus correcting for presence of endogenous creatinine.

Fourteen dilutions of PAH, ranging in concentration from 75 to 1050 mg. %, were further diluted by a factor of one to twenty and determinations of PAH concentration were made. A treated water blank was again used and zero concentration was represented by the reading

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*(to correspond to values expected in urine samples)

of a diluted urine sample.

(v) Measurement of urine concentration of creatinine and PAH: From ten minute urine volumes below 2 ml., between 2 and 6 ml., between 6 and 10 ml., and above 10 ml., 0.5, 1.0, 2, or 3 ml. of urine respectively was diluted to 100 ml. in a volumetric flask. One-ml. aliquots were then diluted to 10 ml. and to 20 ml. for determination of creatinine and PAH respectively, using the procedures outlined above.

Solute Content of Urine and Plasma

Depression of the freezing point of a solution is proportional to the number of solute particles present in the solution. The solute concentration is usually expressed in osmoles per liter. (One osmole per liter corresponds approximately to one gm. molecular weight of non-ionized solute dissolved in 1000 gm. of water.)

An Aminco apparatus was used to determine freezing point depression. Compressed air is allowed to expand in the cooling chamber of this unit resulting in uniform supercooling of the test sample, which is stirred by a glass-enclosed thermistor probe. Temperature changes, converted to resistance changes by the thermistor, are observed by means of an indicator tube which is balanced with a variable resistor. At the freezing point (melting point) of the sample the temperature of the solution remains constant for an appreciable length of time. The balance reading in arbitrary resistance units at this point is recorded.

Standard solutions of sodium chloride ranging in concentration from 100 to 1850 milliosmoles per kg. of water were prepared, and a relation between osmolality and resistance at the freezing point was obtained. A standard curve was prepared and used to determine the concentration of solute in plasma and urine samples.

Determination of Urinary Sodium and Potassium

All urine samples, diluted one to fifty with distilled water, were analyzed for sodium and potassium content by the use of the Beckman DU Flame Spectrophotometer. Appropriate dilutions of an artificial stock urine containing 250 m Eq./l. of sodium, 200 m Eq./l. of potassium, and 20 gm./l. of urea were made to obtain ten solutions ranging in concentration from 25 to 250 m Eq./l. of sodium and from 20 to 200 m Eq./l. of potassium. Standard curves were drawn relating concentration to per cent transmittance and used to determine the sodium and potassium content of urine samples.

Accuracy of Experimental Methods

Evans blue dye determinations: The standard deviation of the dye concentration differences of twenty random pairs of samples was found to be $\pm$ 0.04 x 10^{-6} gm./ml. The error due to sampling is not included in this calculation.

Renal functions: Twenty pairs of duplicate samples of urine and plasma were carried through the procedures for creatinine and PAH.

Standard deviations of the difference between pairs of samples and average per cent differences between samples are shown below:

	Plasma creat.	Urine creat.	Plasma PAH	Urine PAH
S.D. (mg.%)	± 0.1	± 30.9	± 0.02	± 5.6
Av. % diff.	1.8	3.4	2.4	2.7

Osmolality: Ten samples of Ringer-Locke's solution were tested over a period of four months and the standard deviation from the average concentration was found to be ± 2 m Osm./l.

Sodium and Potassium Determinations: The standard deviation of ten determinations of the same sample of urine was ± 5 m Eg./l.

Statistical Analysis of Results

An analysis of variance of the split-plot type was used to determine significance among treatments (hydrated, normal, and dehydrated infusions; hydrated infusions, exchanges, and controls) and among readings (control, infusion, and post-infusion periods). Least significant differences were calculated in order to determine which treatments and readings were significantly different from the others. The sources of variation and the degrees of freedom for the analysis used are shown below:

Source	D.F.
Treatments	2
$Error_{(1)}$	27
$Total_{(1)}$	29
Readings	2
Readings x Treatments	4
$Error_{(2)}$	54
Total	89

RESULTS

Preliminary Experiments

Infusions of homologous donor blood in ten anesthetized dogs resulted in variable diuretic responses. An attempt was made to obtain an objective criterion of diuresis by relating urine flow increase in different animals to some function of their body weight. If renal response does take part in the regulation of intravascular volume it would seem reasonable to relate change in urine flow (V) to initial blood volume. Consequently the "urine flow index" was derived:

$$UF_i = \frac{(\text{Total V over 1 hr. after inf.}) - (\text{Tatal V over 1 hr. before inf.})}{\text{initial blood volume}} \times 100$$

Arbitrarily letting a UF_i of 2.0 or higher represent a "good" diuresis, only four animals of the above series showed a large diuretic and natriuretic response. A second series of nine dogs received infusions of autologous blood which had been withdrawn from each animal two weeks prior to use and stored in the cold in one-third strength "ACD" solution. Only two of these infusions resulted in marked diuresis and natriuresis.

Expected hematocrit changes following infusion (obtained from the calculated pre-infusion cell volume plus volume of red cells infused and the measured plasma volume immediately after the infusion) were frequently lower than actual hematocrit measurements in both groups of animals, indicating either a release of red cells trapped in the spleen

NORMAL - INFUSION

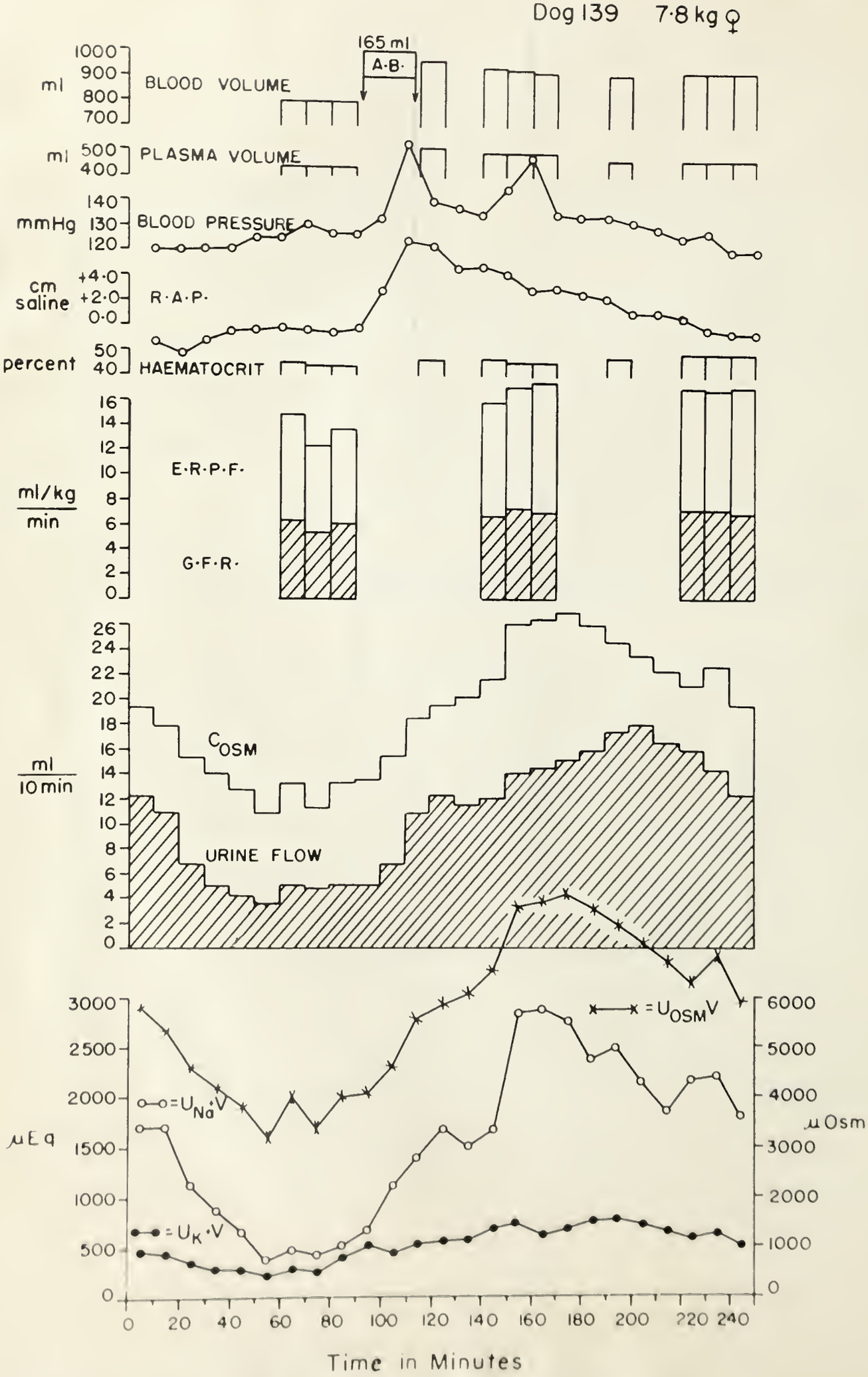


Figure 3: A diuretic and natriuretic response to an infusion of "artificial blood" in a normally hydrated, anesthetized dog. The initial high urine flow was due to a "spontaneous diuresis", which was sometimes observed in this series. Abbreviations are the same as in Fig. 1.

C_{Osm} = volume of urine necessary for excretion of total solute if isotonic urine only were produced.

or a loss of fluid (and probably dyed albumin) from the circulation). Since exchanges of homologous blood for blood from the experimental animal resulted in overt allergic reactions in three out of five cases, and since dye-tinged skin wheals were seen in some animals following the infusion, loss of protein and fluid from the circulation due to increased capillary permeability was thought to be responsible for the inconsistent diuretic response following blood volume expansion. A nonreactive "artificial blood" was therefore used to assess the effect of predictable intravascular volume expansion on kidney function.

Infusion into Differentially Hydrated Dogs

The results obtained from infusion of "artificial blood" into normal, dehydrated, and prehydrated dogs (Series I-III) were analyzed in the following manner: measurements of different variables during the three main experimental periods (Fig. 1) were tabulated (Tables I-III, Appendix). An analysis of variance was carried out on selected parameters as described earlier under "Methods" and the statistical significance of changes between experimental periods in each series of animals and between corresponding periods in different series of animals was determined. Changes from control to infusion periods in sets of variables which might be expected to be causally related were analyzed by using linear regression analysis to draw graphical lines of best fit and by determining correlation coefficients for the sets of variables to obtain a measure of the statistical significance of the relationships.

DEHYDRATED – INFUSION

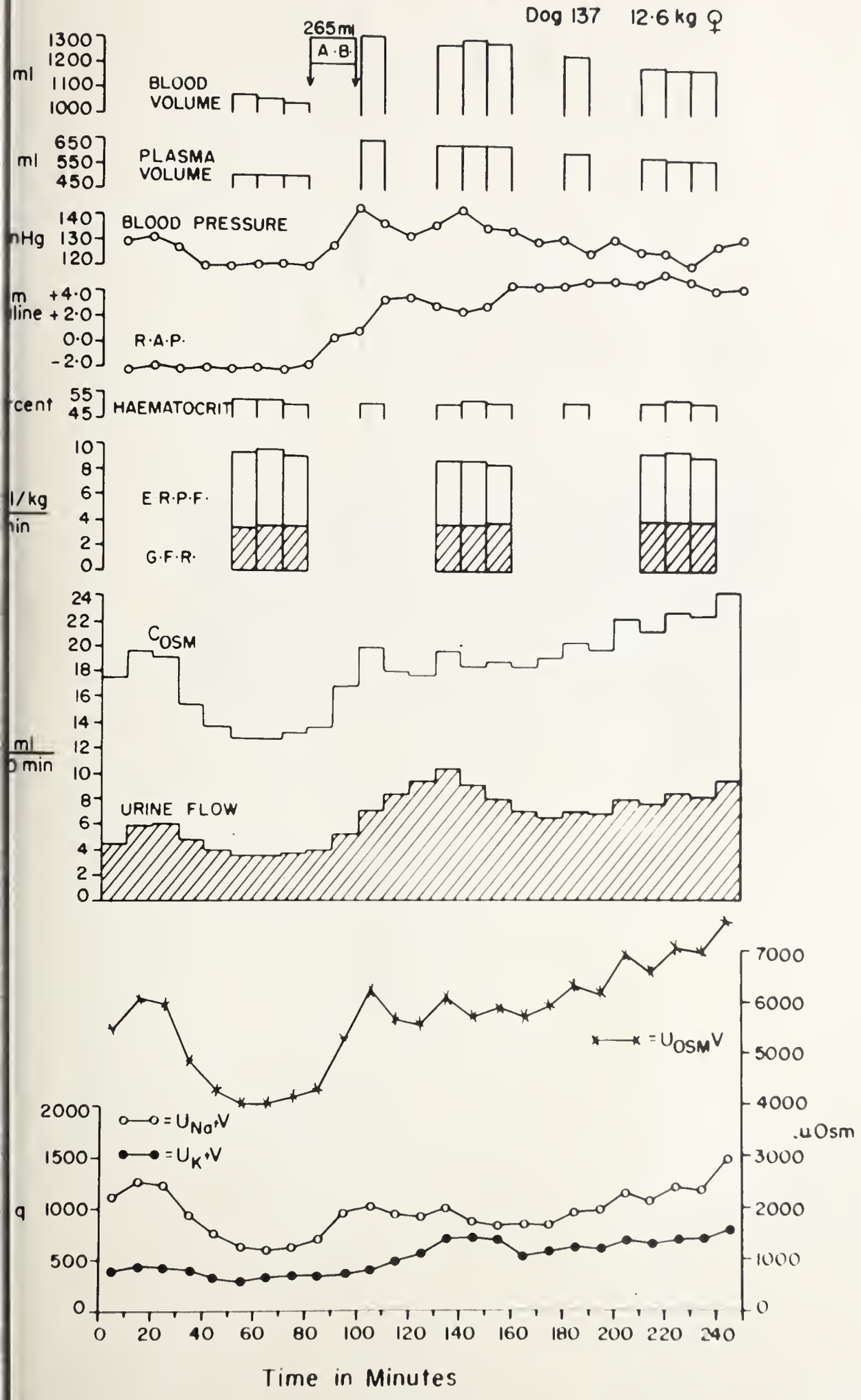


Figure 4: Renal response to infusion in an anesthetized, dehydrated dog. Scales and abbreviations are the same as in Fig. 3. Note the smaller diuretic response and, in contrast to Fig. 3, the decrease in E.R.P.F. following infusion.

1. Comparisons within groups

a) "Normal infusions": Many of the changes which were found to be significant in this group are seen in the plot of a representative experiment (Fig. 3). Plasma and blood volumes consistently increased following the infusion and returned towards control levels in the post-infusion period. However, since a different method was used to determine plasma volume in the earlier experiments of this series, the statistical significance of the changes was not determined. Right atrial pressure uniformly rose following infusion, a finding common to Series I to III. Urine flow showed a highly significant (p=less than 0.01) increase from control to infusion period and this increase was maintained in the post-infusion period. A similar maintained, highly significant increase in urinary solute excretion was observed, which was due mainly to sodium and to some extent to potassium, since the increases in these ions followed the same pattern as the changes in total solute excretion, significance being found at the 1% and 5% level of probability for sodium and potassium rise respectively. Increase in glomerular filtration rate following infusion was highly significant and a further significant (p-less than 0.05) increase occurred from infusion to post-infusion periods. Effective renal plasma flow rose throughout the experiments, showing a significant increase over control only in the third period.

b) "Dehydrated infusions": Fig. 4 is a plot of a representative experiment of this group. The rise in plasma volume following infusion

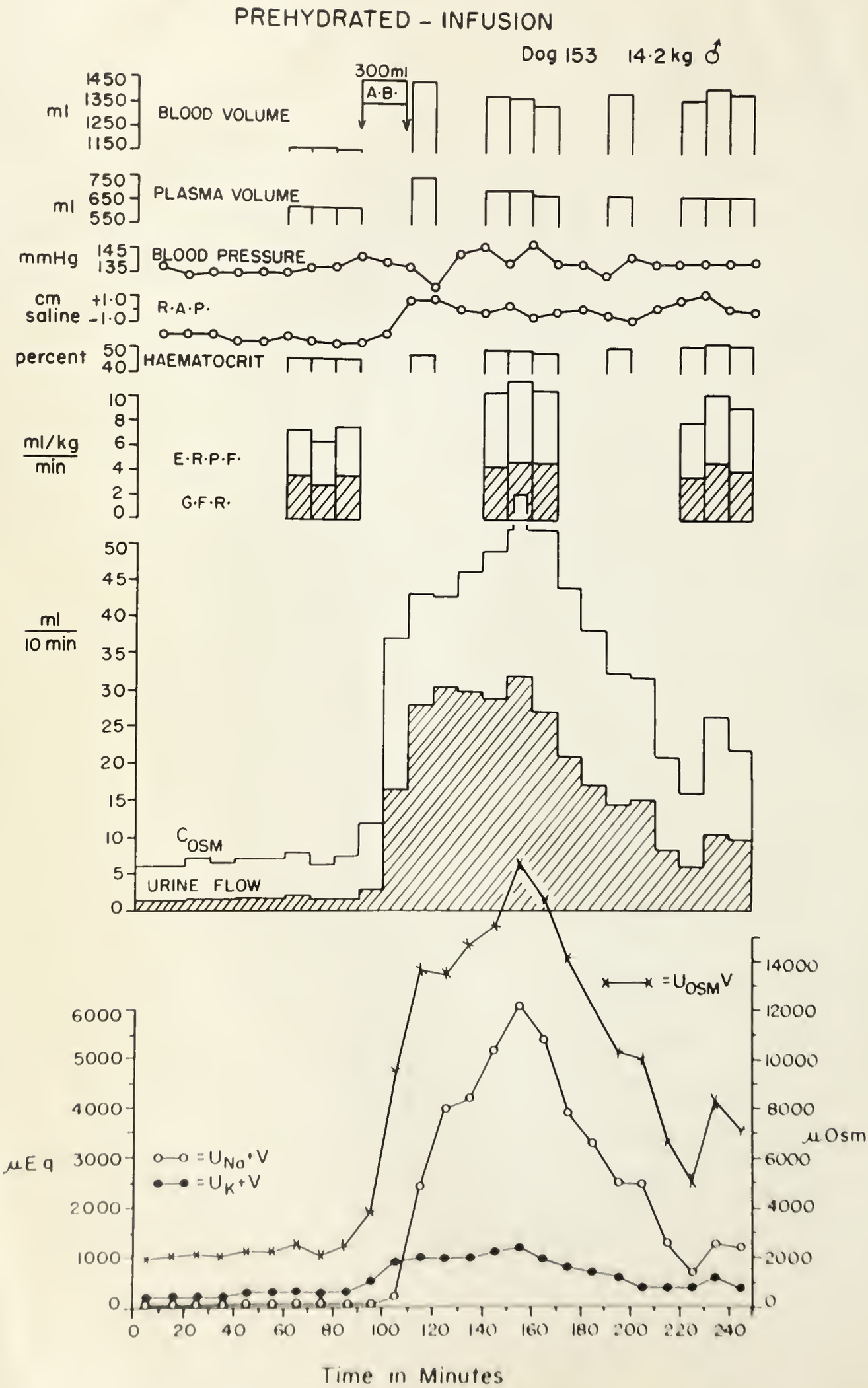

PREHYDRATED - INFUSION
Dog 153 14·2 kg ♂
300ml
A·B·
ml
1450
1350
1250
1150
BLOOD VOLUME
ml
750
650
550
PLASMA VOLUME
mmHg
145
135
BLOOD PRESSURE
cm saline
+1·0
-1·0
R·A·P·
percent
50
40
HAEMATOCRIT
ml/kg/min
10
8
6
4
2
0
E·R·P·F·
G·F·R·
ml/10 min
50
45
40
35
30
25
20
15
10
5
0
C_{OSM}
URINE FLOW
$= U_{OSM}V$
μEq
6000
5000
4000
3000
2000
1000
0
$= U_{Na^+}V$
$= U_{K^+}V$
14000
12000
10000
8000
6000
4000
2000
0
μOsm
0 20 40 60 80 100 120 140 160 180 200 220 240
Time in Minutes

Figure 5: A marked diuretic and natriuretic response in an animal orally prehydrated with saline. Note the compressed scales for urine flow and sodium, potassium, and solute excretion.

in this series was highly significant and the decrease in the post-infusion period also was significant at the 1% level. Blood volume changes were similar to those of plasma volume, but no statistical significance was found for these differences. Urine flow again increased (p=less than 0.01) following the infusion and a further slight increase was observed in the post-infusion period. The excretion of solute rose significantly during the infusion period and a further rise resulted in a highly significant difference in solute output between control and post-infusion periods. Changes in sodium excretion were similar, and the output was significantly different from control during the third experimental period. Urinary potassium rose significantly on infusion, a further slight increase being observed towards the end of the experiment. While G.F.R. changes and levels of significance were comparable to those found in series I, E.R.P.F. uniformly decreased upon infusion, returning towards normal in the following period. The slight fall did not result in a statistical difference between control and infusion values.

c) "Prehydrated infusions": This group showed the most marked diuretic and natriuretic response to blood volume expansion as illustrated by the representative experiment (Fig. 5). The increase in plasma volume (p=less than 0.01) following infusion was maintained in the third experimental period, as was the significant increase in blood volume. Urine output during the infusion period was larger (p = less than 0.01) than during the control and post-infusion periods, although the increase

from control to post-infusion was still highly significant. Urinary solute excretion and urinary sodium and potassium output showed the same changes as urine flow, except that only 5% levels of significance were found for potassium excretion. G.F.R. changes were comparable to those in Series I and II. A slight, statistically insignificant rise in E.R.P.F. during the infusion period was observed in this group.

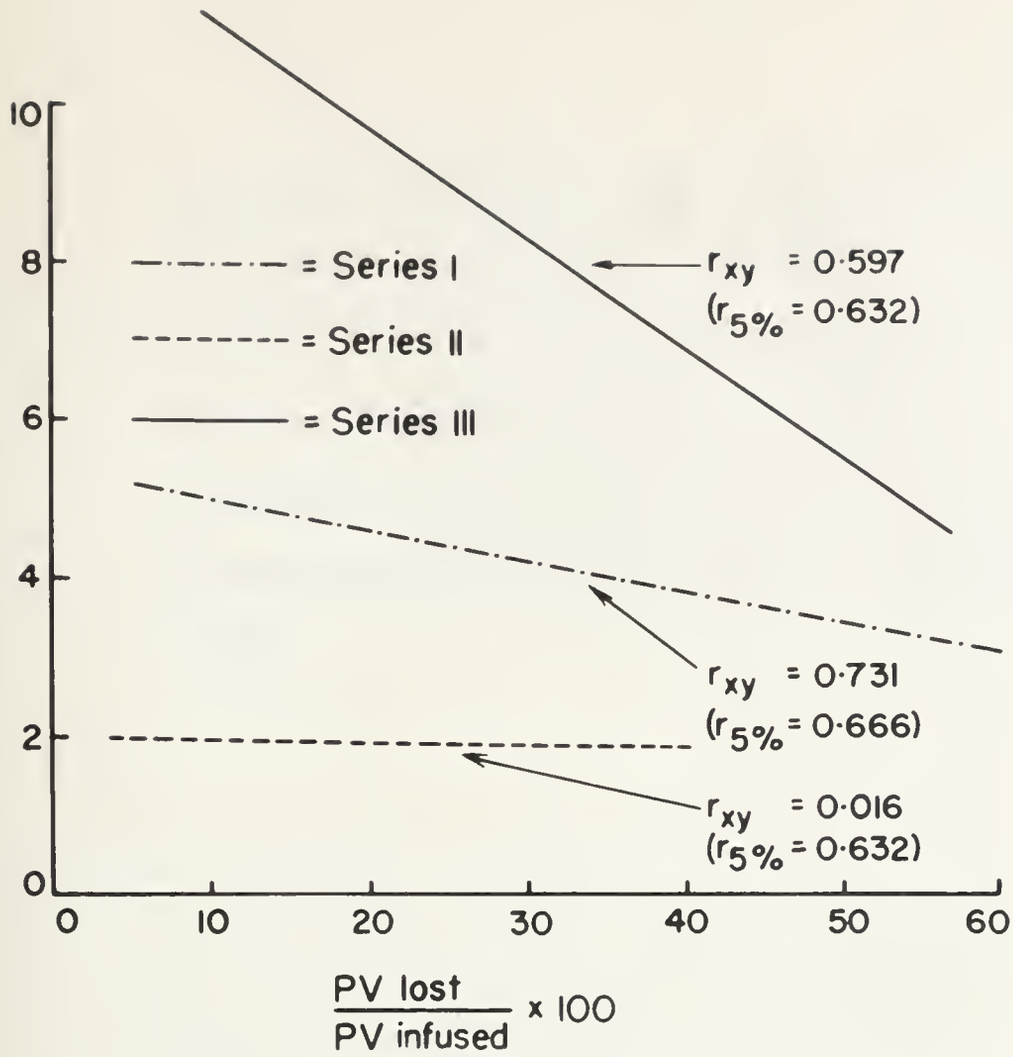
2. Comparisons among groups

No significant difference in control plasma and blood volumes was found between the three groups of animals. Comparable increases in these variables following infusion were maintained during the post-infusion period in prehydrated but not in dehydrated dogs. The decrease in volume in Series I from infusion to post-infusion periods could be traced to the earlier method of plasma volume measurement. Later determinations using the modified method indicated some maintenance of plasma and blood volume increases in this group also.

The total urine production of Series II was significantly below that of the other two series. Urine volume during the control period was larger in Series I than in Series II and III, the difference being significant in the case of the dehydrated animals. Urine flows in prehydrated dogs during the infusion period were significantly higher than in the normal group of dogs and a highly significant difference was found between levels in these animals and the lower urine flows of the dehydrated dogs during this period. Post-infusion volume of urine in Series II was significantly lower than the corresponding volumes in Series I and III.

Total solute excretion of the dehydrated animals was lower (p = less than 0.05) than the excretion of either the normally hydrated or the prehydrated dogs. Output during the control period was highest in the normal group (p = less than 0.05). Excretions during the infusion period were all significantly different at the 1% level, the largest value occurring in the prehydrated series and the smallest appearing in the dehydrated series. During the post-infusion period the level of solute excretion of the normally hydrated animals was above that of the other two groups, the difference between normal and dehydrated dogs being highly significant.

Differences between groups similar to those of urine flow and total solute output were found for sodium excretion, dehydrated animals having a lower (p = less than 0.01) total excretion than the other two series. No statistically significant difference was found between control values, although the same relative differences were observed as for control urine flows. During the infusion period the prehydrated animals showed the largest increase in sodium output, followed by that of the normal group, the dehydrated group again giving the least response. These differences were significant at the 1% level. A maintained high level of sodium excretion which was significantly above the corresponding levels of Series I and II was seen in Series I during the third experimental period. The normally hydrated group also excreted significantly more potassium than either of the other two groups, due mainly


10
8
6
4
2
0
= Series I
= Series II
= Series III
r_{xy} = 0·597
($r_{5\%}$ = 0·632)
r_{xy} = 0·731
($r_{5\%}$ = 0·666)
r_{xy} = 0·016
($r_{5\%}$ = 0·632)
0
10
20
30
40
50
60
PV lost / PV infused x 100

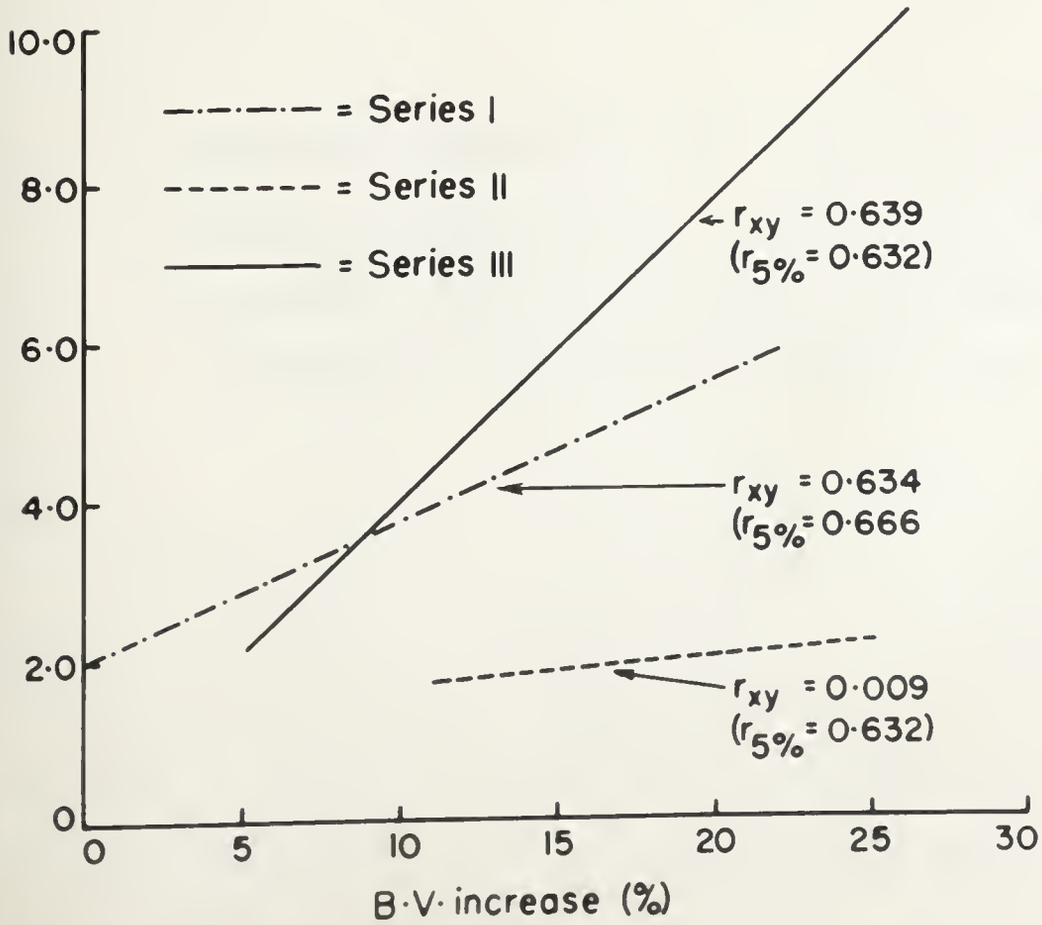

10·0
8·0
6·0
4·0
2·0
0
= Series I
= Series II
= Series III
r_{xy} = 0·639
($r_{5\%}$ = 0·632)
r_{xy} = 0·634
($r_{5\%}$ = 0·666
r_{xy} = 0·009
($r_{5\%}$ = 0·632)
0
5
10
15
20
25
30
B·V· increase (%)

Figure 6a: Correlation of proportion of infused plasma lost to the interstitium with urine flow index in series I to III. Note different levels of statistical significance. The value of the correlation coefficient (rxy) compared to the value at the 5% level of statistical significance ($r_{5\%}$) indicates the degree of "scatter" in each series.

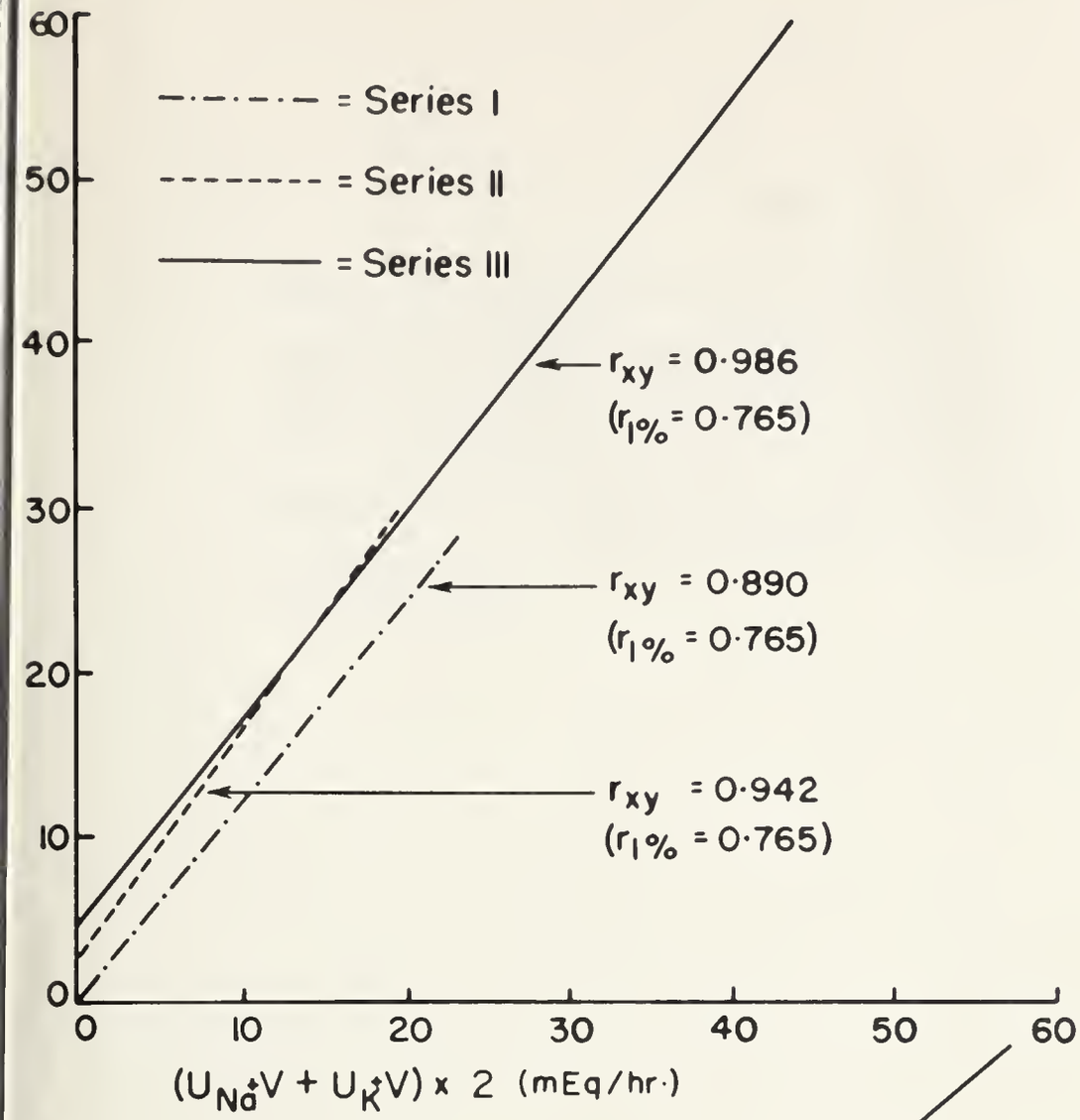
Figure 6b: Correlation of immediate increases of blood volume following infusion with urine flow index in series I to III. In contrast to normal and prehydrated animals note lack of correlation in dehydrated dogs.

to a significantly higher control level of output, since a rise in potassium excretion following infusion was noted in all series (p = less than 0.05). The increase in urinary potassium from control to infusion periods of the dehydrated group was also significantly lower than the corresponding increases in the other series. In addition a statistically significant difference in this parameter between normal and dehydrated animals was seen in the post-infusion period.

As mentioned above, G.F.R. rose in the majority of animals regardless of the state of hydration. The increase from control to infusion periods was significant at the 1% level, while the further increase from infusion to post-infusion periods was significant at the 5% level. There were no statistically significant differences in control values of E.R.P.F., although values in the normal group were generally above those of either of the other groups. However, following infusion E.R.P.F. levels (which fell instead of rose) in dehydrated animals were significantly lower than corresponding levels in Series I and III, p-values being less than 0.01 and 0.05 for differences from normal and prehydrated groups respectively.

3. Correlation between changes in variables

As outlined above, the degree of statistical correlation between changes from control to infusion periods of selected pairs of parameters was determined. No relationship was found to exist between the small, inconsistent variations in plasma osmotic pressure and the increases in urine flow following infusion of artificial blood. The changes in urine flow with right atrial pressure also did not show a significant relation


= Series I
= Series II
= Series III
r_{xy} = 0·986
($r_{1\%}$ = 0·765)
r_{xy} = 0·890
($r_{1\%}$ = 0·765)
r_{xy} = 0·942
($r_{1\%}$ = 0·765)
0
10
20
30
40
50
60
$(U_{Na^+}V + U_{K^+}V) \times 2$ (mEq/hr·)

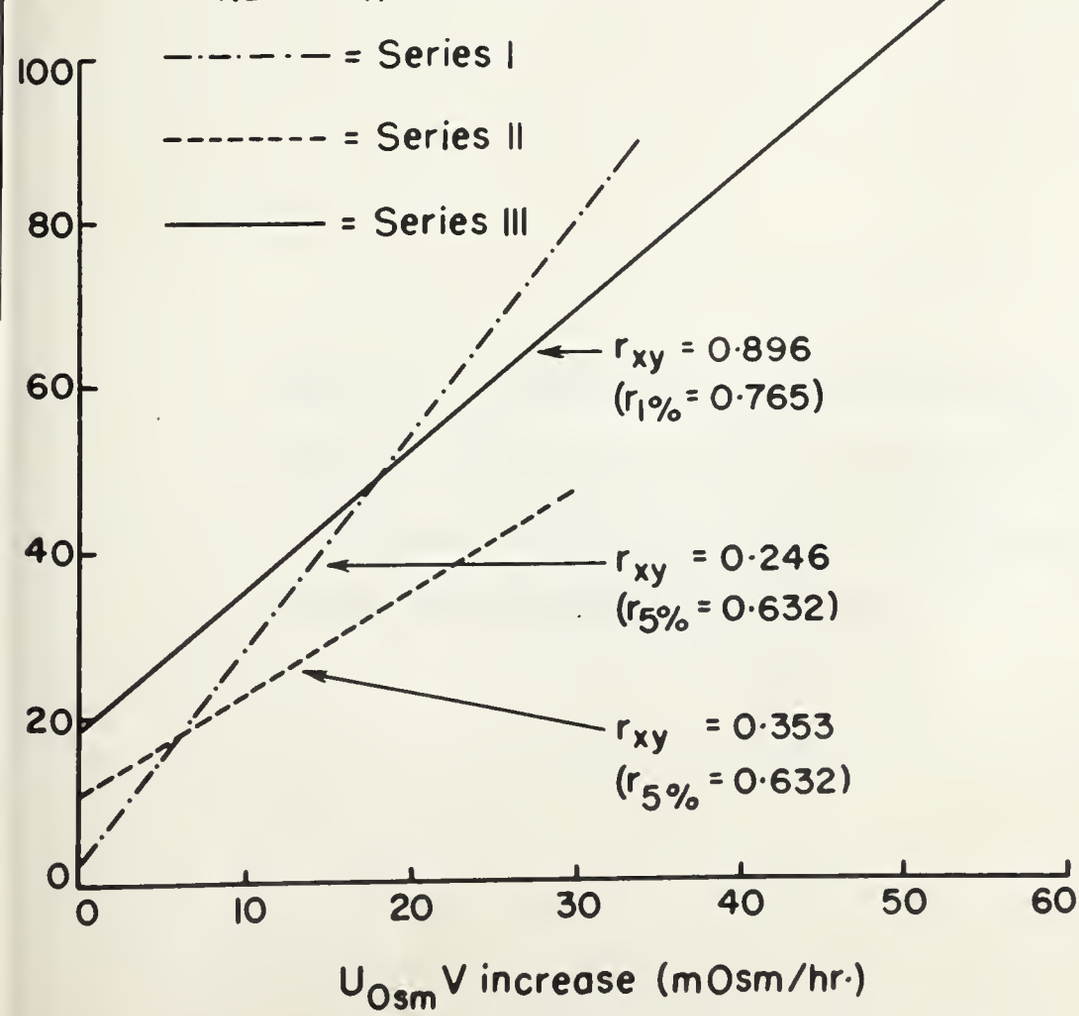

= Series I
= Series II
= Series III
r_{xy} = 0·896
($r_{1\%}$ = 0·765)
r_{xy} = 0·246
($r_{5\%}$ = 0·632)
r_{xy} = 0·353
($r_{5\%}$ = 0·632)
0
20
40
60
80
100
$U_{Osm}V$ increase (mOsm/hr·)

Figure 7a: Relation of increase from control to infusion periods in combined sodium and potassium excretion to the corresponding increase in total solute output in series I to III. Note the excellent correlation obtained in each group.

Figure 7b: Relation of increase from control to infusion period in total solute output to increase in urine flow in differentially hydrated dogs. Significant correlation is seen in prehydrated animals only.

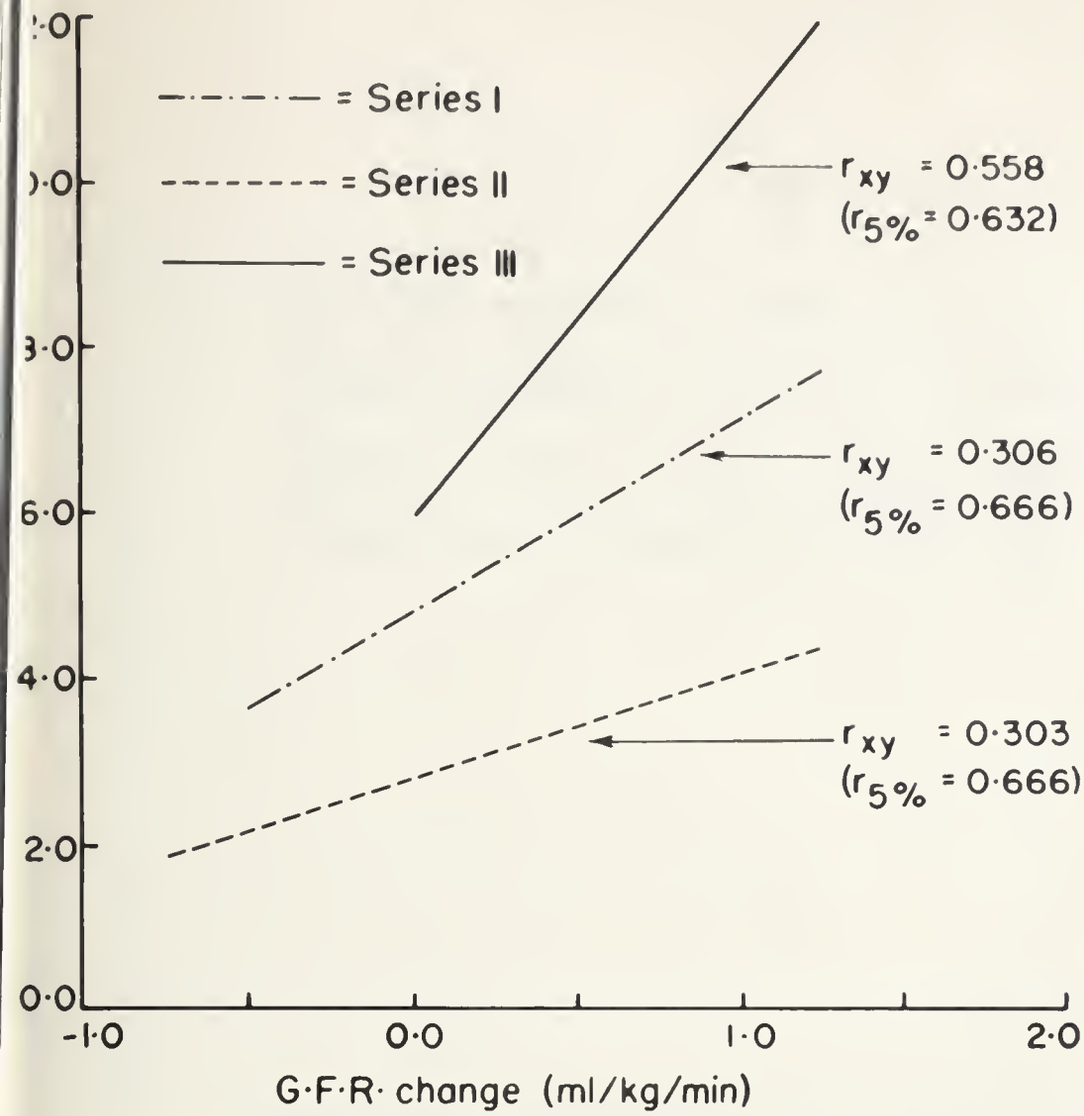
although a positive trend was observed in normal and prehydrated animals.

Correlation between per cent increase in blood volume and urine flow index in the different groups is illustrated in Fig. 6b). A significant positive correlation was found in Series III, and a trend approaching the 5% level of statistical significance was seen in the normal group. No relation between blood volume expansion and urine flow change was observed in the dehydrated animals, however. The difference in slope between Series I and III indicates a larger diuretic response to comparable volume expansion in prehydrated dogs.

Fig. 6a) demonstrates the relationship between volume of infused fluid lost to the interstitium and urine flow index. A significant negative correlation was found in the group of normally hydrated animals and a similar negative correlation in the prehydrated group approached the 5% level of significance. Again no significant trend was seen in dehydrated dogs.

A highly significant relationship between changes in combined sodium and potassium excretion and increase in total urinary solute output was seen in series I to III (Fig. 7a). Comparing solute output to urine flow increase (Fig. 7b), highly significant correlation was obtained in prehydrated animals only, while the trends in the other two series were not statistically significant.

Changes in G.F.R., related to urine flow are presented in Fig.


= Series I
= Series II
= Series III
r_{xy} = 0·558
($r_{5\%}$ = 0·632)
r_{xy} = 0·306
($r_{5\%}$ = 0·666)
r_{xy} = 0·303
($r_{5\%}$ = 0·666)
0·0
2·0
4·0
6·0
-1·0
0·0
1·0
2·0
G·F·R· change (ml/kg/min)

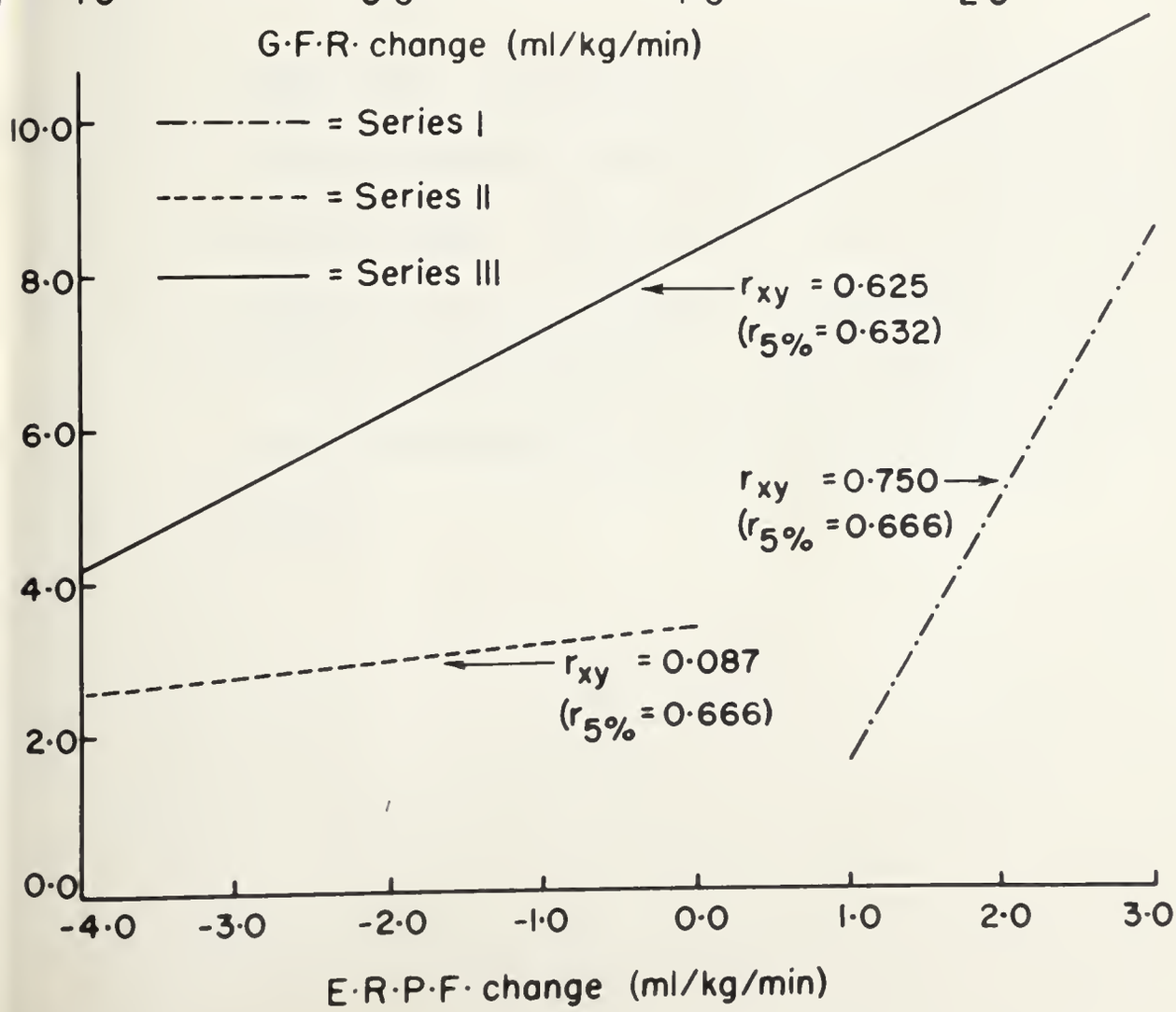

= Series I
= Series II
= Series III
r_{xy} = 0·625
($r_{5\%}$ = 0·632)
r_{xy} = 0·750
($r_{5\%}$ = 0·666)
r_{xy} = 0·087
($r_{5\%}$ = 0·666)
0·0
2·0
4·0
6·0
8·0
10·0
-4·0
-3·0
-2·0
-1·0
0·0
1·0
2·0
3·0
E·R·P·F· change (ml/kg/min)

Figure 8a: Correlation between urine flow increase and change in glomerular filtration rate following infusion (series I to III). Only in series III does the relationship approach statistical significance.

Figure 8b: Correlation between urine flow increase and change in effective renal plasma flow in differentially hydrated dogs. Note that while both increases and decreases in E.R.P.F. are seen in prehydrated animals, normal dogs show increases only, while dehydrated animals uniformly responded to infusion with a decrease in E.R.P.F.

8a). No statistical significance was found, although the correlation coefficient for changes in prehydrated animals did approach a p-value of 5%. The variation of urine flow increase with effective renal plasma flow changes shows marked differences between groups (Fig. 8b). While both increases and decreases in E.R.P.F. were seen in the prehydrated group, only increases were observed in normal animals following the infusion. Conversely, consistent decreases of E.R.P.F. were found in the series of dehydrated dogs. The positive correlation with urine flow of the normal group was significant at the 5% level, while that of prehydrated animals approached this level of significance.

Infusion, Exchange, and Control Experiments in Prehydrated Dogs

Different experimental treatments were compared in prehydrated dogs (Series III to V). Various parameters were again measured and tabulated (Table III to V, Appendix), analysis of variance and correlation were carried out as before.

1. Comparisons within groups

a) Prehydrated infusion: Infusion of artificial blood resulted in highly significant increases of plasma and blood volume, which were maintained in the post-infusion period. Similar highly significant, maintained increases were seen in urine flow, total solute excretion, and renal sodium output. The sustained rise in potassium excretion (p = less than 0.05) was not dependent on the treatment (infusion, exchange, or control),

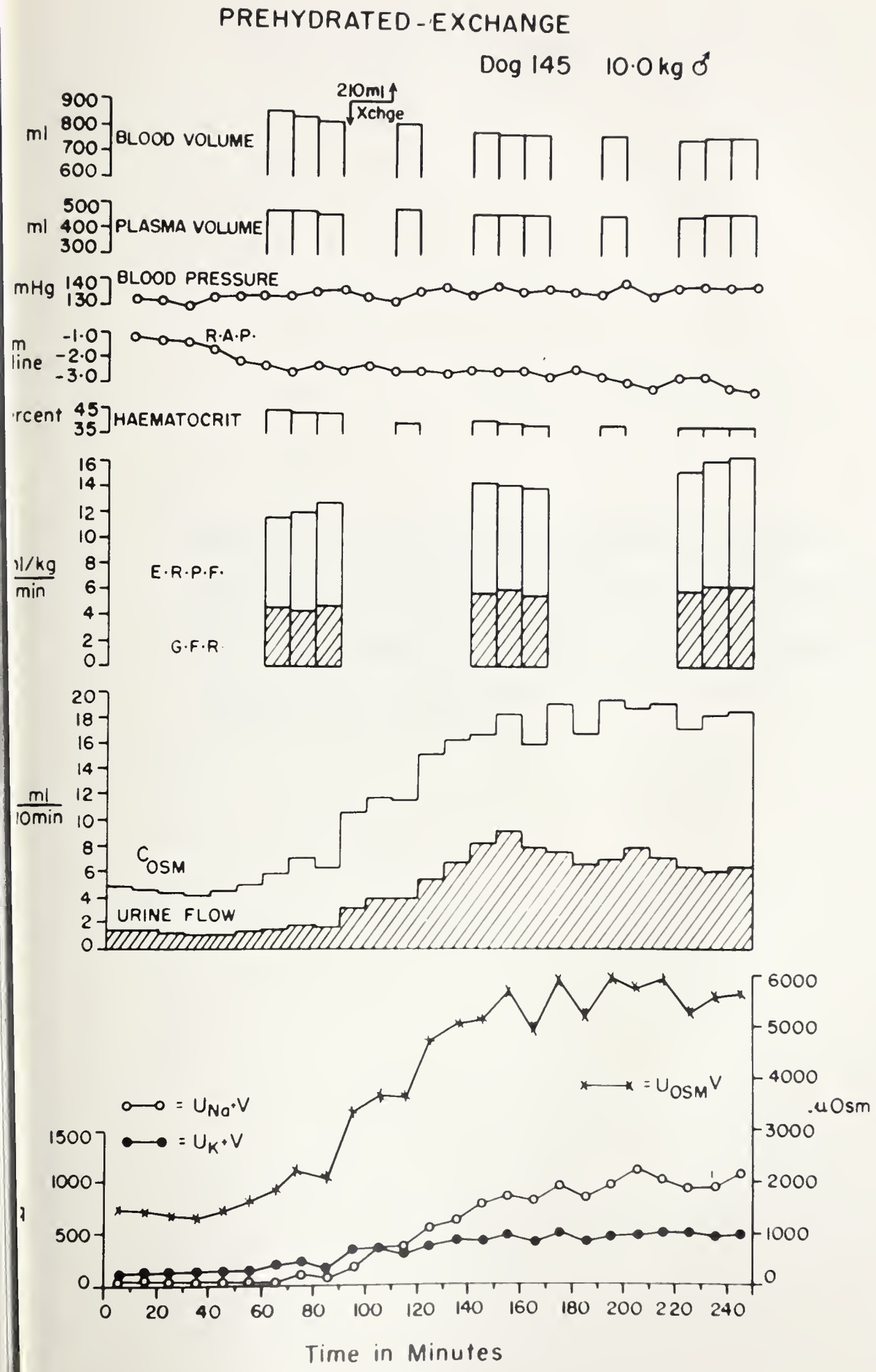

PREHYDRATED-EXCHANGE
Dog 145 10·0 kg ♂
210ml
Xchge
ml
900
800
700
600
BLOOD VOLUME
ml
500
400
300
PLASMA VOLUME
mHg
140
130
BLOOD PRESSURE
m
line
-1·0
-2·0
-3·0
R·A·P·
rcent
45
35
HAEMATOCRIT
ml/kg
min
16
14
12
10
8
6
4
2
0
E·R·P·F·
G·F·R·
ml
10min
20
18
16
14
12
10
8
6
4
2
0
C OSM
URINE FLOW
= U Na·V
= U K·V
= U OSM V
1500
1000
500
0
6000
5000
4000
3000
2000
1000
0
.uOsm
0
20
40
60
80
100
120
140
160
180
200
220
240
Time in Minutes

Figure 9: Representative experiment of series IV (isovolemic exchange). Note the increase in urine flow and solute excretion despite the relative constancy of plasma and blood volumes.

since comparable changes were observed in each of the three groups of animals.

The increase in G.F.R. from control to infusion and post-infusion periods was statistically significant at the 1% level. Significance (p = less that 0.05) also was found for the rise in E.R.P.F. following the infusion.

b) "Prehydrated exchange": In expected contrast to the infusions no significant changes were found to occur in either plasma and blood volumes or right atrial pressures (Fig. 9). Urine flow increase following the isovolemic exchange of artificial blood was significant at the 5% level. A statistically insignificant decrease was seen from infusion to post-infusion periods.

The same changes, with the same levels of statistical significance were observed in total solute excretion. While sodium output also rose slightly during the infusion period, no significance was found for this change.

G.F.R. increase following exchange was highly significant and the higher level of filtration rate was maintained in the post-infusion period. A slight, statistically insignificant rise in E.R.P.F. was followed by a return to normal levels by the end of the experiment.

c) "Prehydrated control": Similarly to the series of exchanges, no significant differences in plasma and blood volume or right atrial pressure were observed in the control experiments (Fig. 10). A highly

PREHYDRATED – CONTROL

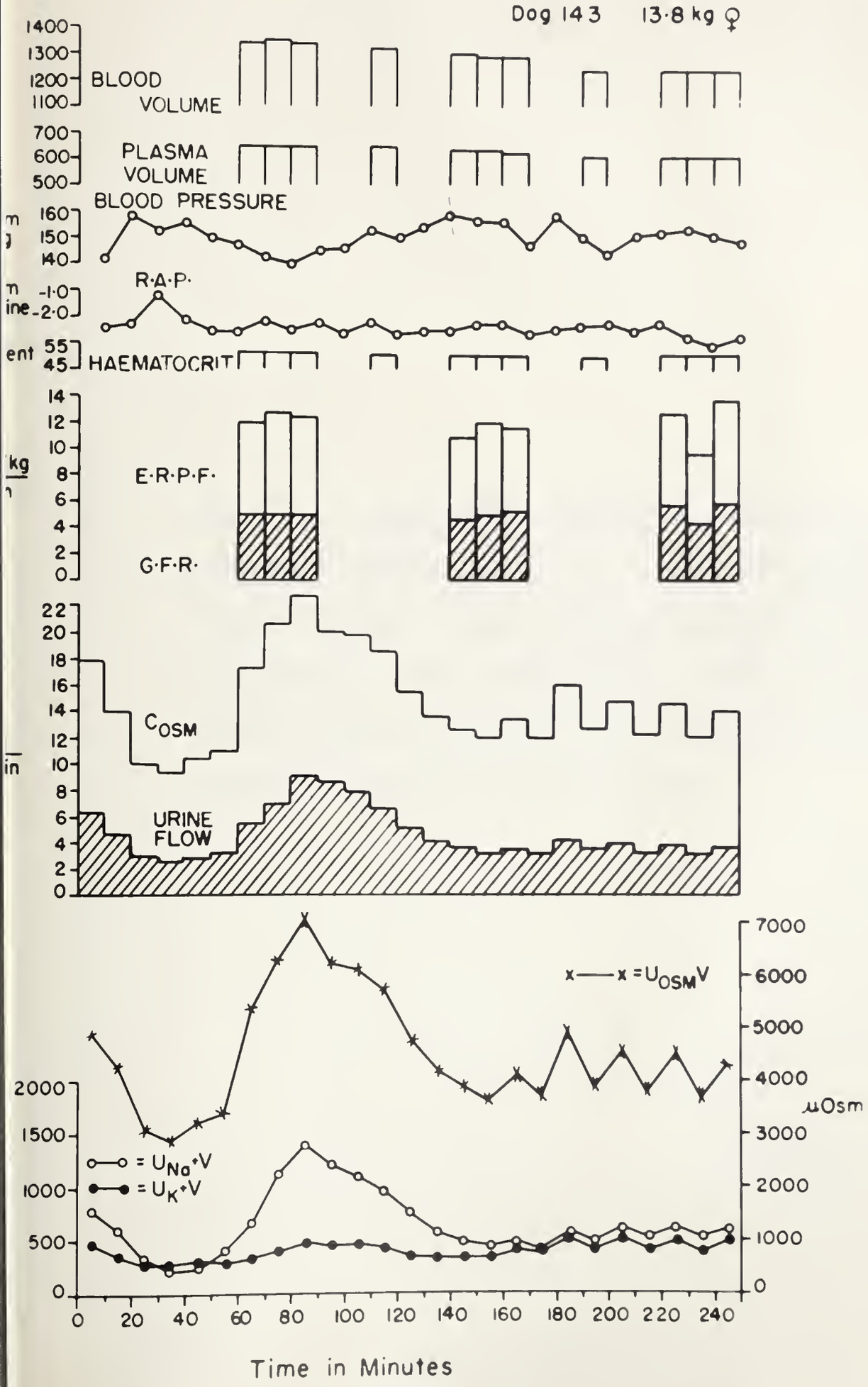


Figure 10: Representative experiment of the group of prehydrated control (series V). A small spontaneous diuresis is shown, which is associated with increases in sodium and total solute excretion. Note the typical feature that the diuresis precedes the period normally following infusion in the other series.

significant, maintained decrease in urine flow was observed, associated with comparable changes in both total solute and sodium excretion (p = less than 0.01). This unexpected finding was probably due mainly to three "spontaneous" diureses, which occurred during the first period in this series. No significant change in G.F.R. was found, while decrease in E.R.P.F. from control to infusion and post-infusion periods was significant at the 1% level.

2. Comparisons among groups

Initial plasma and blood volumes of these animals were comparable, and only infusion resulted in a maintained, highly significant increase in intravascular volume.

Total urine production of Series III was markedly above that of Series IV and V (p = less than 0.01). Urine flows during the control period were significantly higher in Series V than corresponding volumes in either of the other two groups. During the infusion period the large increase in Series III was significantly different at the 1% level from values in Series IV and V, while significant difference at the 5% level was found between urine volumes of the exchange and control groups. In the post-infusion period the level of urine flow of the group of prehydrated infusions remained above that of the exchange or control group (p = less than 0.01).

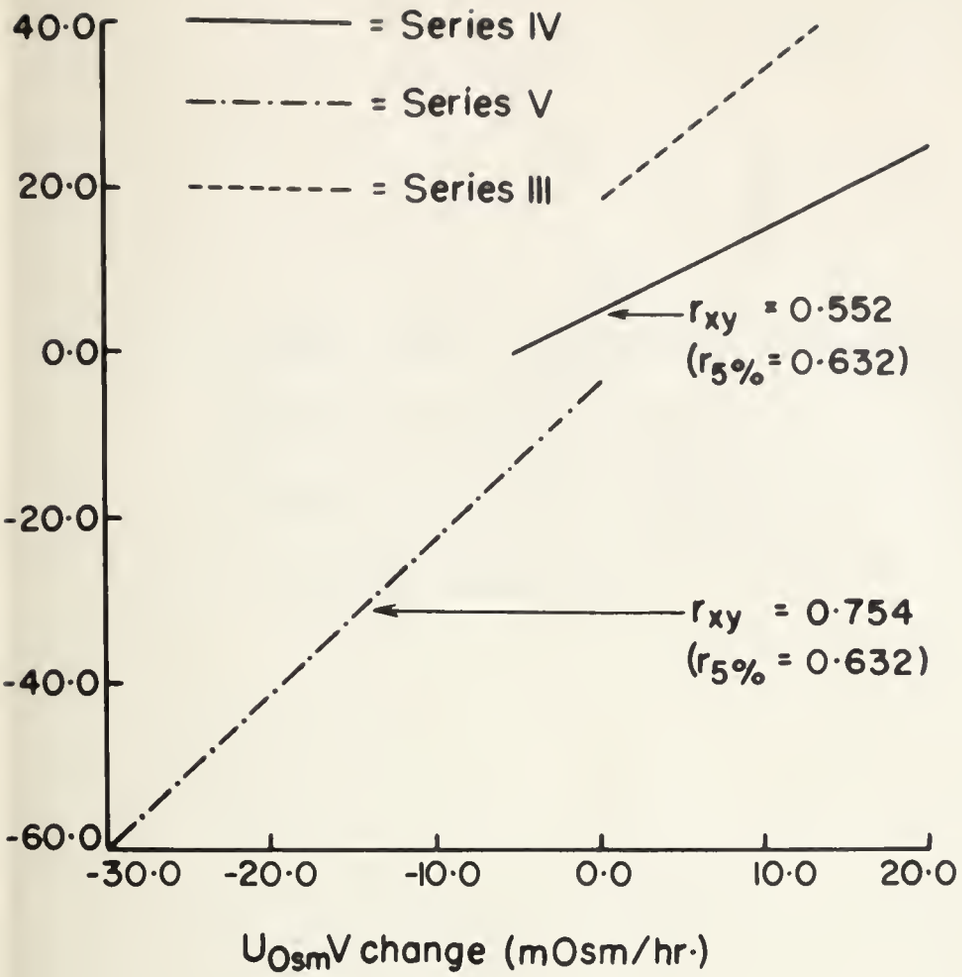
Total solute excretion was parallel to changes in urine flow and the same levels of statistical significance were found for the differences

between groups. Similar variations in sodium output were observed, except that no significant difference was seen between infusion periods of the series of exchanges and controls. As mentioned above, no statistically significant difference in potassium output was found between groups.

During the control period glomerular filtration rates of the hydrated control animals were higher than the corresponding values in Series III and IV (p = less than 0.01). In the infusion period G.F.R. of Series III was increased significantly over Series IV and V. No statistically significant differences were found between filtration rates during the third experimental period. Similarly to G.F.R., the initial levels of renal plasma flow were significantly higher in Series V than in the other two groups of animals. Although lower E.R.P.F. values in the exchanges than in the infusion series were found during the infusion period, this difference was not statistically significant. A highly significant difference was seen between infusion and control series during this period, however. No significant differences in E.R.P.F. were seen between groups in the post-infusion period.

3. Correlation between changes in variables

Lines of best fit were again determined by the method of linear regression and correlation coefficients were calculated. In Series IV no correlation was found between fluid loss from the interstitium and urine flow increase. Although G.F.R. increase in this series was highly


= Series IV
= Series V
= Series III
40·0
20·0
0·0
-20·0
-40·0
-60·0
r_{xy} = 0·552
($r_{5\%}$ = 0·632)
r_{xy} = 0·754
($r_{5\%}$ = 0·632)
-30·0
-20·0
-10·0
0·0
10·0
20·0
$U_{Osm}V$ change (mOsm/hr·)

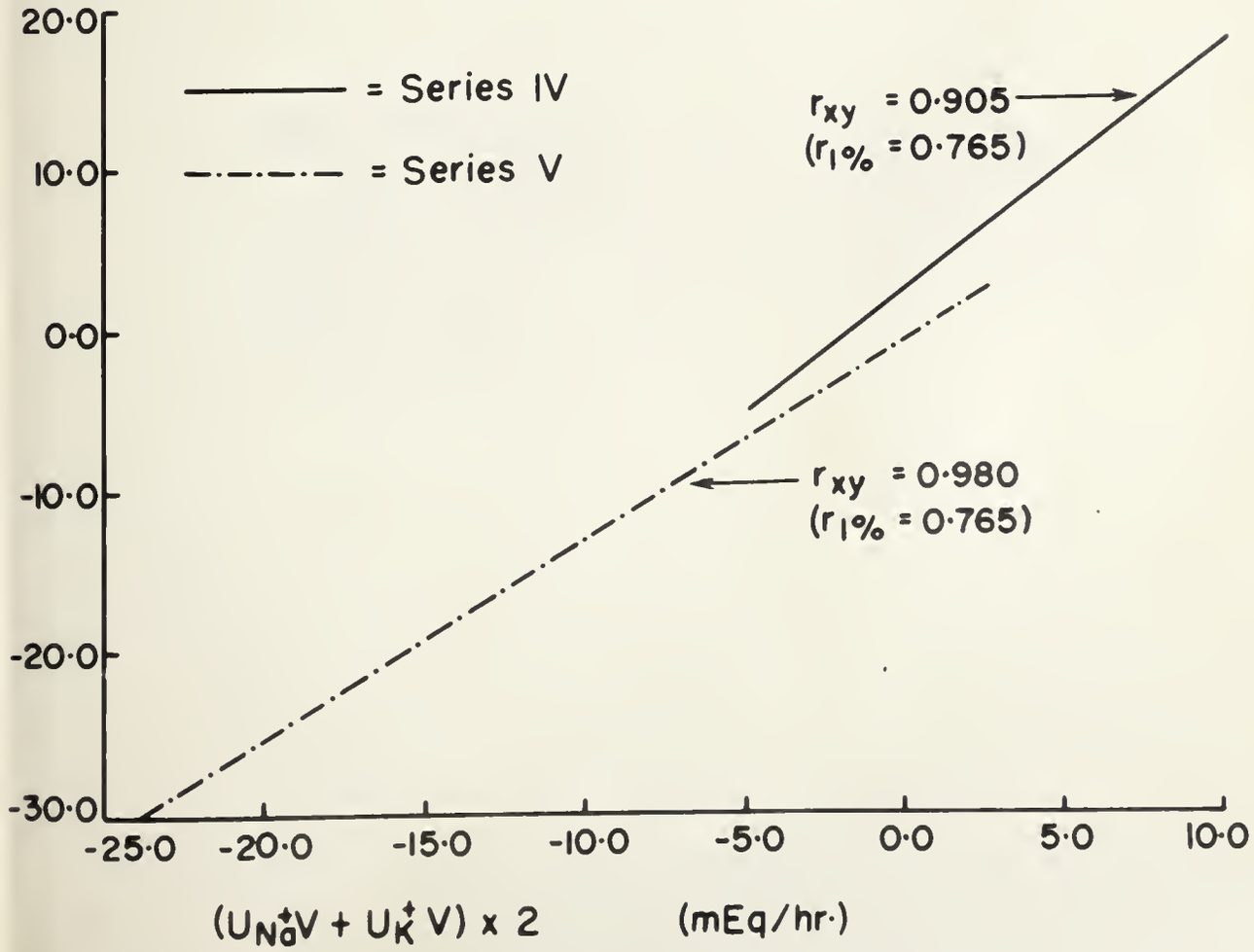

= Series IV
= Series V
20·0
10·0
0·0
-10·0
-20·0
-30·0
r_{xy} = 0·905
($r_{1\%}$ = 0·765)
r_{xy} = 0·980
($r_{1\%}$ = 0·765)
-25·0
-20·0
-15·0
-10·0
-5·0
0·0
5·0
10·0
$(U_{Na^+}V + U_{K^+}V) \times 2$ (mEq/hr·)

Figure 11a: Relationship between changes in urine flow and solute output from control to infusion periods in series III to V. Correlation coefficient for series III is given in Fig. 7b.

Figure 11b: Change in urinary solute output compared to change in combined sodium and potassium excretion (series III to V). The line of Series III coincides with that of series IV.

significant it was not quantitatively associated with the increase in urine flow, since no relationship between the two changes was observed. The variation of E.R.P.F. with urine flow changes did, however, show significant positive correlation. The decrease in urine flow of Series V from control to infusion periods was associated with decreases in both G.F.R. and E.R.P.F. which approached the 5% level of significance.

Fig. 11a) demonstrates the relationship between urine flow and solute output changes in the three series. Correlation in control animals was found to be significant, and a positive trend approaching statistical significance was seen in the exchange series.

Highly significant relationships were again observed between changes in combined sodium and potassium excretion and total solute output (Fig. 11b).

In all of these experiments (Series I to V) the calculated osmolar clearance, that is the volume of urine necessary to excrete urinary solutes if isotonic urine only were produced, was at a higher level than the observed urine flow, indicating a consistent production of hypertonic urine.

DISCUSSION

The purpose of the reported experimental work was to obtain a quantitative relationship between intravascular volume expansion and renal response in differentially hydrated dogs, to gain information on the mechanism of this response, and to assess renal effects of the infusate other than those due to blood volume increase.

By means of comparison of renal functions in prehydrated control dogs with renal functions in prehydrated animals receiving an isovolemic exchange of artificial blood, it was hoped to separate spontaneous variations from changes due to the infusate. Unfortunately, the significantly higher urine flow of the control group during the first experimental period (due mainly to three "spontaneous" diureses) tends to invalidate a comparison of these two series. However, since no diuresis was observed during the second experimental period in any of the control animals, and since a slight fall in urine flow occurred even when no spontaneous diuresis was seen during the control period, the increases in urine flow and to some extent in solute output following exchange probably represent changes in renal function due to the infusate. Lack of any consistent relationship of intravascular volume changes, differences in interstitial fluid volume, and variations in plasma osmolarity to diuresis eliminates changes in these variables as causative factors in the observed increase in urine flow. Therefore a physiological or pharmacological effect of artificial blood on the kid-

ney or a dilution of circulating hormones must be supposed.

The mechanism by which the infusate influences kidney function can be investigated by relating changes in renal functions and by comparing these changes to corresponding variations in the control series. Although a significant rise in G.F.R. following exchange was observed, the total lack of correlation with increases in urine flow tends to eliminate the factor of increased filtered load as the cause of diuresis. In addition, while no statistically significant change in G.F.R. was found in the control animals, increases of comparable magnitude were frequently observed, sometimes even associated with decreases in urine flow. In view of these findings alteration of tubular function is the most probable mechanism of diuresis in the exchange series. Dilution of antidiuretic hormone and consequent alteration in tubular reabsorption of water would result in increased urine production. Since absence of ADH also leads to increased elimination of urea (Ullrich, 1960), and since potassium excretion increased in prehydrated animals independent of the treatment(due possibly to prolonged anesthesia) the increase in total solute output following exchange might be expected. The fact that no statistically significant increase in urinary sodium was found supports this hypothesis.

The magnitude of diuresis following infusions of artificial blood, combined with the marked increase in sodium output, leaves no doubt that possible dilution effects of the infusate at best contribute only slightly to the observed renal response. Since diuresis and natriuresis

could also not be explained on the basis of the inconsistent changes in plasma osmolarity and interstitial fluid volume, the response must be due to expansion of intravascular volume. The results obtained confirm earlier findings (Pearce, 1959, Atkins and Pearce, 1959) that increased blood volume results in enhanced secretion of sodium as well as of water. In normal and prehydrated animals the positive correlation of blood volume with urine flow tends to support the theory of Gauer and Henry that hypervolemia initiates diuretic response. The theory is not supported, however, by such a relation in the group of dehydrated animals.

The significant differences in total solute and water excretion between groups of differentially hydrated dogs must be due to the operation of a mechanism separate from that of blood volume expansion, since infusion of artificial blood resulted in comparable increases in intravascular volume. No significant differences were found in initial blood volumes between groups. The maintenance of blood volume in different states of hydration may be explained on the basis of fluid shifts between intravascular and interstitial compartments, as suggested by the Starling-Landis hypothesis and by the work of Walker, Ross and Hammond. From these findings it may be concluded that initial interstitial fluid volume modifies the renal response to blood volume expansion.

If it is accepted that regulatory fluid shifts do occur between the two extracellular compartments, the work of Bálint and Pethes on

the renal effects of rapid saline infusion in prehydrated and dehydrated concious dogs confirms the role of interstitial fluid volume in modifying renal response. In agreement with results obtained in this laboratory, these investigators observed a larger increase in urine flow in prehydrated than in dehydrated animals following the infusion. However, while hypertonic urine was produced in the dehydrated series, animals which had been loaded with 30 to 40 ml. of water or saline 8 to 14 hours prior to the experiment eliminated hypotonic urine following infusion of saline. No marked difference in sodium output was observed between the two groups of animals. Those aspects of their findings which conflict with results reported here may be explained if it is remembered that anesthesia has a potentiating effect on ADH secretion (Smith, 1951). The wellknown fact that saline infusion in dogs results in consistent increase in G.F.R. suggests that the natriuresis in both groups of dogs, which responded to infusion with comparable large increases in creatinine clearance, was due to a somewhat different mechanism from that initiating increased sodium excretion following infusion of an iso-oncotic blood volume expander.

The preceding considerations are consistent with the mechanism of renal response proposed by Henry and Gauer, who suggested that discharge from volume receptors reflexly inhibited secretion of ADH. This mechanism must, however, be modified by hypothesizing an effect of interstitial fluid volume on the release of antidiuretic hormone. An additional action on solute excretion is postulated, since statisti-

cally significant differences in solute output following infusion were seen between differentially hydrated animals. A possible mechanism of natriuresis is an increase in filtered load. However, Atkins and Pearce found no consistent changes in G.F.R. following infusion of plasma which resulted in diuresis and natriuresis. In agreement with this finding, no correlation between solute output and filtration changes was obtained in the reported experiments on normal and dehydrated dogs. Since a significant relationship between urine flow and solute excretion was also absent, the conclusion of Atkins and Pearce that a tubular mechanism independent of that for water excretion is reponsible for enhancing sodium output seems applicable. In the prehydrated group, however, variation of G.F.R. with urine flow approached statistical significance, and the correlation of filtration rate with combined sodium and potassium output was statistically significant ($r_{xy} = 0.742$; $r_{5\%} = 0.632$). This finding may be related to the work of Mertz (1960). Comparing the level of urine flow and sodium excretion in variously hydrated human subjects he found significant correlation between extracellular fluid volume and glomerular filtration rate. Natriuresis was also related to G.F.R. Mertz concluded that increased water elimination in his experiments was due to the operation of the Henry and Gauer mechanism and that co-ordination between the impulses of the volume receptors and those which are initiated by hypothetical receptors regulating G.F.R. might partly explain the natriuresis of volume expansion. Returning to the

present experiments, since the rise in G.F.R. was not significantly different between series, and since no correlation between filtration changes and urine flow or solute excretion was found in normal and dehydrated animals, increase in filtered load may contribute to diuresis and natriuresis in prehydrated animals only and other influences of interstitial fluid volume on excretion of salt and water following blood volume expansion must still be assumed to explain all of the observed results.

The differences in effective renal plasma flow between groups can be explained on the basis of changes in inhibition of ADH. Hilger, Kluemper and Ullrich (1958) found that the permeability of collecting ducts to both water and urea was increased by antidiuretic hormone. The recent work of Jaenike (1961) on the equilibration of C^{14} - labelled urea between medullary interstitium and fluid from collecting ducts confirms these results. In the absence of A.D.H., urea, which normally contributes largely to the osmotic pressure in the renal medulla is excreted in greater amount. The resulting decrease in medullary solute content, reflected in a decrease of viscosity of blood in this region, will allow an accelerated flow of blood through the vasa recta and, if changes in medullary blood flow are associated with corresponding changes in E.R.P.F., lead to increases in effective renal plasma flow during inhibition of A.D.H. The correlation of E.R.P.F. with urine flow in the series of exchanges is also consistent with the above interpretation. Increase in renal blood flow as a causative factor of

diuresis is unlikely, since diuresis despite a fall in E.R.P.F. was observed in dehydrated and occasionally in prehydrated animals. The consistent decrease in renal plasma flow following infusion in dehydrated dogs was a striking finding. This decrease was unassociated with urine flow and occurred in spite of increases in G.F.R. A constricting effect on efferent arterioles is proposed, the mechanism of which remains unclear, however.

Since the natriuresis of hypervolemia can not be explained on the basis of renal hemodynamic changes, the operation of another hormonal mechanism also influenced by interstitial fluid volume is postulated. Inhibition of aldosterone would provide a logical mechanism for the natriuretic response. It has been shown, however, that adrenalectomy does not abolish the increase in sodium output following infusion of plasma (Atkins and Pearce, 1959). In addition, while aldosterone is believed to initiate exchange of sodium for potassium, thus associating antikaluresis with natriuresis, the significant increases in potassium excretion in prehydrated, normal, and dehydrated dogs also suggest that aldosterone inhibition is not the mechanism of increased solute output. A hormonal influence inhibiting active solute reabsorption, as suggested by different workers, is the probable explanation for the observed natriuresis. A hormonal inhibition of solute reabsorption could also explain the relationship of G.F.R. to solute excretion seen only in prehydrated animals. Since filtration rate increased significantly in all three series reabsorption of most of the filtered load must have

occurred in normal and dehydrated dogs. As release of the proposed hormone is assumed to be promoted by an increased interstitial volume, a relationship between G.F.R. and solute output in the prehydrated series of dogs might be expected.

In summary, a theory relating intravascular volume expansion and interstitial fluid volume to renal response in solute and water excretion is proposed, which offers an explanation for the results obtained in this investigation and is compatible with findings of other workers. Depending on the state of tissue hydration, expansion of blood volume results in inhibition of antidiuretic hormone and a release of a hypothetical "saluretic" hormone promoting tubular solute loss. Information from intravascular volume receptors is integrated with information from postulated receptors located in the interstitial fluid space. A decreased interstitial volume results in inhibition of renal response to blood volume expansion and an increased interstitial volume enhances the response. This hypothesis provides a mechanism for fluid retention despite intravascular volume expansion following periods of reduced fluid intake and for enhanced elimination of solutes and water following periods of excess intake.

CONCLUSIONS

1. In the reported experiments on blood volume expansion in differentially hydrated, anesthetized dogs the infusate used is believed to have been without direct effect on renal function, since

the small diuresis following isovolemic exchange can be explained on the basis of dilution of A.D.H.

2. This dilution effect does not explain the magnitude of diuresis and natriuresis following expansion of intravascular volume.

3. The mechanism of renal response is unlikely to be hemodynamic, since changes in both G.F.R. and E.R.P.F. can be explained on a different basis.

4. Evidence is presented for the existence of separate hormonal mechanisms controlling excretion of water and solutes and is related to the hypothesis of Gauer and Henry as modified by Atkins and Pearce.

5. The level of tissue hydration plays an important role in determining the magnitude of diuresis and natriuresis of intravascular volume expansion.

6. The mechanism of the contribution of interstitial fluid volume to regulation of blood volume is believed to consist of central integration of information from hypothetical interstitial receptors with impulses from intravascular volume receptors.

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APPENDIX

ABBREVIATIONS

Av. P.V.	= average plasma volume
Av. B.V.	= average blood volume
B.V. increase	= immediate per cent increase in blood volume over control levels
A.B. infused	= volume of artificial blood infused
A.P. infused	= volume of artificial plasma infused
A.P. lost	= immediate expansion of recipient plasma volume subtracted from volume of A.P. infused
P_{Osm} change	= change in plasma osmolarity of recipient from control to infusion period
Av. R.A.P.	= average right atrial blood pressure
Av. B.P.	= average arterial blood pressure
Urine Flow	= total volume of urine produced in each experimental period
G.F.R.	= average glomerular filtration rate during the second half of each experimental period
E.R.P.F.	= average effective renal plasma flow during the second half of each experimental period
$U_{Na^+}V$	= total renal excretion of sodium during each experimental period in milli equivalents
$U_{K^+}V$	= total renal excretion of potassium during each experimental period in milli equivalents
$U_{Osm}V$	= total renal solute excretion during each experimental period in milli osmoles
C_{Osm}	= osmolar clearance
UF_i	= urine flow index
C.I.P.	= control, infusion, and post-infusion period respectively

(Table IV)

Net plasma removed	= plasma volume infused subtracted from plasma volume withdrawn
Fluid lost from interstitium	= immediate change in recipient plasma volume added to net plasma removed

Table I - NORMAL INFUSION

Dog No.		Av.P.V. (ml)	Av.B.V. (ml)	B.V. increase (%)	A.B. infused (ml)	A.P. infused (ml)	A.P. lost (ml)	P_{Osm} change (m Osm)	Av. R.A.P. (cmH_2O)	Av.B.P. (mmHg)	Urine flow (ml)	G.F.R. ($\frac{ml/kg}{min}$)	E.R.P.F. $\frac{ml/kg}{min}$	$U_{Na}+V$ (mEq)	U_k+V (mEq)	U_{OsmV} (mOsm)	C_{Osm} (ml)	UF_i
114	C	648	1514						-1.1	168	19.2	7.64	18.72	3.75	3.75	30.74	99.2	
	I	717	1560	5.0	265	165	108	-5	+1.3	169	103.7	7.62	22.05	13.53	6.63	63.30	204.1	5.6
15.3 kg	P	571	1262						-0.2	166	75.3	6.61	24.03	15.42	4.44	60.20	194.4	
115	C	606	1063						-1.8	134	12.4	7.22	13.55	0.52	2.40	27.77	58.0	
	I	610	1071	7.2	145	97	30	0	-0.2	134	57.4	7.89	17.43	5.52	3.78	36.00	117.9	4.2
9.0 kg	P	493	895						-0.6	138	44.3	8.26	15.82	5.13	2.82	33.94	110.7	
116	C	542	896						-1.7	120	19.2	4.07	8.08	3.00	3.06	23.44	73.8	
	I	505	840	-2.9	175	104	141	0	+0.1	120	30.4	5.12	9.80	7.20	3.51	34.38	108.2	1.2
12.0 kg	P	429	735						-1.6	120	36.2	5.17	10.90	10.56	3.15	38.65	124.2	
117	C	472	927						-0.2	139	86.4	5.41	15.95	5.28	5.82	30.11	98.4	
	I	489	960	6.3	180	112	89	-/5	+4.5	123	90.2	5.22	16.22	11.19	4.83	41.68	136.2	0.4
10.5 kg	P	385	745						-1.8	128	71.2	5.66	17.90	11.01	4.05	42.97	140.8	
119	C	437	995						-0.6	113	46.3	6.48	13.05	5.79	3.90	30.45	97.1	
	I	473	1103	12.6	240	141	75	+2	+1.6	106	76.0	6.08	14.22	11.28	4.44	44.88	141.9	3.0
11.2 kg	P	-	-						-	-	-	-	-	-	-	-	-	
131	C	502	940						-5.3	121	19.8	4.83	10.85	3.42	1.53	21.45	69.3	
	I	531	1025	14.4	186	97	34	-3	+0.4	138	82.0	5.97	13.63	6.84	3.87	37.83	125.1	6.6
9.0 kg	P	510	985						-1.9	124	45.5	6.26	13.82	5.16	3.87	38.11	124.9	
138	C	565	1003						-4.5	118	36.7	5.08	11.05	2.85	2.16	23.14	74.6	
	I	627	1169	21.6	210	105	5	+5	-1.0	130	92.9	5.97	13.00	7.38	3.42	34.37	111.0	5.6
10.0 kg	P	-	-						-3.0	124	54.8	6.69	14.20	9.24	3.66	42.50	137.2	
139	C	441	800						-0.4	125	27.6	6.03	13.73	3.36	1.74	22.60	75.5	
	I	504	940	21.0	165	91	15	0	+4.3	142	75.3	7.13	16.93	11.85	3.66	39.65	132.3	6.0
7.8 kg	P	470	916						+0.3	125	94.3	7.12	17.13	12.69	3.87	40.02	133.4	
140	C	387	833						-4.8	144	20.1	4.32	12.57	3.30	1.50	22.08	72.4	
	I	511	1070	29.0	220	120	0	0	-1.5	146	18.5	5.23	14.07	2.25	2.40	22.24	73.0	-0.2
10.2 kg	P	475	980						-2.8	143	22.9	5.62	16.30	5.10	2.55	26.74	87.7	
141	C	537	1078						-3.3	136	20.7	-	-	1.92	1.65	20.53	67.4	
	I	628	1248	19.7	225	122	9	-5	+1.0	136	71.8	-	-	7.35	3.03	39.86	132.9	4.7
10.5 kg	P	612	1231						-0.3	137	48.6	-	-	5.22	2.25	32.25	107.7	

Table II - DEHYDRATED INFUSION

Dog No.		Av. P.V. (ml)	Av. B.V. (ml)	B.V. incr. (%)	A.B. inf. (ml)	A.P. inf. (ml)	A.P. lost (ml)	P_{Osm} change (mOsm/l)	Av. R.A.P. (cmH_2O)	Av. B.P. (mmHg)	Urine Flow (ml)	G.F.R. (ml/kg/min)	E.R.P.F. (ml/kg/min)	$U_{Na}{}^{+}V$ (mEq)	$U_k{}^{+}V$ (mEq)	$U_{Osm}V$ (mOsm)	C_{Osm} (ml)	UF_i
118	C	608	1177						+0.2	133	6.1	4.23	-	0.19	1.20	15.16	48.0	
	I	638	1271	14.3	210	125	48	-13	+1.3	146	23.1	4.85	-	2.52	1.98	27.92	88.5	1.4
12.2 kg	P	548	1097						+1.6	142	25.8	4.43	-	1.56	2.19	25.72	81.3	
128	C	448	949						-2.5	124	12.8	4.08	11.48	1.89	1.68	17.73	55.4	
	I	470	986	11.7	150	84	28	- 2	+0.6	129	23.9	4.87	11.42	2.73	3.12	26.07	81.9	1.2
9.5 kg	P	483	1010						-0.2	126	34.7	5.43	12.62	7.56	2.91	36.88	114.3	
129	C	506	1045						-4.2	119	11.1	4.35	13.93	0.30	2.19	16.42	51.6	
	I	592	1214	20.6	250	137	15	+3	-1.1	135	18.5	4.10	12.33	0.69	2.94	18.90	58.6	0.7
13.2 kg	P	-	-						-1.9	126	29.6	4.70	15.88	5.22	3.09	32.02	102.5	
130	C	478	1004						-5.2	136	14.7	4.91	14.03	1.50	1.83	18.16	58.2	
	I	530	1146	18.2	175	94	29	+6	-1.7	143	21.8	5.87	13.73	1.86	1.95	23.53	74.4	0.7
9.1 kg	P	508	1104						-2.6	139	28.0	6.37	14.33	3.60	2.28	29.72	95.1	
132	C	501	845						-0.5	148	11.1	4.41	12.70	0.45	1.38	14.18	45.9	
	I	543	986	23.0	215	116	32	-5	+2.2	157	31.0	3.60	8.86	3.30	1.92	23.95	77.3	2.4
10.0 kg	P	500	920						+0.7	142	25.3	5.38	11.83	2.94	2.82	25.39	82.0	
133	C	513	1125						-2.1	129	29.1	3.45	11.70	3.27	2.28	25.21	79.3	
	I	576	1239	12.9	260	144	55	+5	-0.3	130	84.6	3.87	11.45	4.26	2.25	24.35	75.4	4.9
12.2 kg	P	529	1148						+1.1	136	94.3	3.87	10.28	4.65	3.69	28.73	87.2	
134	C	703	1195						-0.3	110	13.8	3.55	9.50	0.81	1.98	16.18	52.8	
	I	789	1439	22.6	285	161	53	+5	+7.3	112	23.1	3.95	8.90	1.32	2.04	23.03	74.2	0.8
13.3 kg	P	705	1292						+6.9	116	26.9	4.80	10.95	1.80	4.86	28.32	91.2	
135	C	580	1330						-6.7	118	7.0	3.52	8.53	0.39	1.47	15.91	50.0	
	I	663	1465	11.3	240	138	33	0	-5.6	124	11.5	3.77	8.02	0.81	1.68	19.68	61.9	0.3
11.0 kg	P	655	1457						-6.1	119	15.0	3.31	7.23	1.98	1.71	18.77	59.1	
136	C	613	1175						-3.8	135	8.5	4.01	11.80	0.27	1.05	10.55	34.1	
	I	695	1362	18.2	230	123	21	0	-1.3	134	59.6	5.27	11.30	7.95	2.67	37.94	120.9	4.4
10.6 kg	P	669	1331						-2.0	127	34.5	5.84	13.72	6.12	2.79	33.15	105.3	
137	C	510	1053						-2.1	121	25.6	3.43	9.35	4.74	2.13	27.13	87.1	
	I	631	1289	24.8	265	144	4	+4	+3.2	136	52.0	3.66	8.56	5.52	3.51	35.08	112.4	2.5
12.6 kg	P	575	1192						+4.7	126	45.7	3.87	9.22	6.48	3.90	39.89	128.4	

Table III - PREHYDRATED INFUSION

Dog No.		Av. P.V. (ml)	Av. B.V. (ml)	B.V. incr. (%)	A.B. inf. (ml)	A.P. inf. (ml)	A.P. lost (ml)	P_{Osm} change (mOsm/l)	Av. R.A.P. (cmH_2O)	Av. B.P. (mmHg)	Urine Flow (ml)	G.F.R. (ml/kg/min)	E.R.P.F. (ml/kg/min)	$U_{Na^+}V$ (mEq)	$U_{K^+}V$ (mEq)	$U_{Osm}V$ (mOsm)	C_{Osm} (ml)	UF_i
144	C	560	1100						-0.2	150	25.6	7.43	23.58	4.14	2.22	25.87	84.9	
	I	630	1280	16.4	225	124	49	-4	+1.7	161	72.6	7.87	22.15	10.92	3.54	46.14	153.6	4.3
10.5 kg	P	625	1280						+1.1	158	58.8	8.21	24.23	8.00	2.70	36.80	122.8	
146	C	587	980						-4.6	102	44.5	6.34	17.20	9.04	3.13	41.35	136.7	
	I	636	1131	15.4	210	116	46	-2	-3.7	112	106.1	7.48	19.05	16.96	3.41	61.12	20.39	6.3
10.0 kg	P	620	1144						-5.1	131	92.8	6.95	17.05	9.60	2.92	38.03	126.9	
152	C	647	1255						-5.1	121	9.0	3.71	8.57	0.41	1.00	11.70	37.9	
	I	673	1363	5.6	270	158	83	-4	-4.0	126	57.6	3.88	9.82	3.58	2.19	26.64	87.0	3.9
12.8 kg	P	805	1605						-4.8	127	17.0	4.06	9.83	0.62	1.04	17.17	54.9	
153	C	627	1163						-2.4	138	9.5	3.30	7.14	0.36	1.56	13.31	42.0	
	I	706	1386	23.8	300	169	44	-3	+0.2	141	177.1	4.50	10.82	28.87	6.22	91.40	290.2	14.4
14.2 kg	P	675	1389						+0.3	140	64.4	3.98	9.17	9.58	2.92	47.18	149.8	
156	C	602	1023						-2.3	117	7.5	3.28	7.00	0.20	1.12	10.41	32.9	
	I	661	1195	19.7	250	132	73	+1	-0.2	120	46.3	4.65	9.10	5.72	3.62	40.07	127.4	3.8
11.8 kg	P	620	1122						-0.1	122	28.2	4.94	9.05	4.67	3.78	31.14	99.3	
163	C	545	975						-2.3	140	15.4	3.18	7.64	2.24	0.90	15.76	49.6	
	I	622	1172	16.7	250	148	71	-2	-1.1	147	113.6	4.37	11.38	13.77	2.82	56.18	177.8	10.1
11.8 kg	P	577	1138						-1.4	153	63.9	3.98	8.68	8.51	2.55	42.97	139.7	
165	C	675	1398						+0.8	125	23.0	4.40	15.48	1.06	2.14	21.49	70.5	
	I	729	1635	10.8	270	158	88	-1	+4.4	132	55.9	4.45	11.12	3.18	1.93	28.07	92.4	2.4
12.8 kg	P	741	1725						+5.4	127	33.3	4.64	11.32	1.26	2.96	28.35	93.6	
171	C	725	1266						-2.6	144	9.3	3.65	9.82	0.46	2.24	16.53	52.7	
	I	820	1498	18.5	250	138	43	-3	-1.6	147	75.1	4.64	13.63	14.38	4.16	62.76	201.8	5.2
11.8 kg	P	786	1469						-1.9	148	30.7	5.26	13.40	6.08	2.46	35.74	116.3	
173	C	558	1077						-4.6	133	8.8	3.95	11.28	0.55	1.50	12.88	43.5	
	I	678	1359	26.2	264	154	17	+9	-1.4	130	105.8	5.47	15.27	17.27	3.98	61.80	204.8	9.0
12.4 kg	P	642	1310						-1.1	136	55.9	5.64	14.32	9.20	3.17	44.52	146.1	
174	C	508	1003						-1.8	122	8.0	2.80	9.03	1.18	0.65	13.18	41.0	
	I	575	1152	18.2	265	151	49	-4	+2.3	127	76.1	2.99	9.21	7.03	1.92	30.93	98.2	6.8
12.5 kg	P	531	1080						+0.6	127	53.8	3.27	9.29	3.90	1.90	22.89	71.8	

Table IV - PREHYDRATED EXCHANGE

Dog No.		Av. P.V. (ml)	Av. B.V. (ml)	B.V. incr. (%)	A.B. exch. (ml)	Net plasma removed (ml)	Fluid lost fr. Interst. (ml)	P_{Osm} Chge (mOsm/l)	Av. R.A.P. (cmH_2O)	Av. B.P. (mm Hg)	Urine Flow (ml)	G.F.R. ($\frac{ml/kg}{min}$)	E.R.P.F. ($\frac{ml/kg}{min}$)	$U_{Na}+V$ (mEq)	$U_k + V$ (mEq)	$U_{Osm}V$ (mOsm)	C_{Osm} (ml)	UF_i
145	C	467	838						-2.2	134	9.4	4.61	12.17	0.24	0.93	10.32	33.0	
	I	465	788	-6.0	205	4	17	-5	-2.5	136	41.3	5.80	14.23	4.02	2.46	28.91	93.9	3.8
10.0 kg	P	464	766						-3.0	138	41.1	6.21	16.05	6.10	2.90	33.85	111.4	
148	C	498	855						-1.7	129	33.4	4.63	12.25	6.66	1.96	32.13	102.4	
	I	537	923	+8.0	245	18	57	-10	-1.4	129	44.4	4.64	12.63	7.72	2.44	39.42	128.9	1.2
11.5 kg	P	554	965						-1.5	133	25.0	4.84	11.60	3.82	2.36	25.53	84.5	
149	C	535	840						-2.6	140	15.6	3.09	8.37	2.34	1.09	18.22	58.8	
	I	519	825	0.0	234	22	22	-5	-2.6	140	27.9	3.57	8.23	3.50	1.80	26.02	85.4	1.5
11.0 kg	P	483	761						-3.1	144	37.9	3.77	8.47	4.28	2.72	33.58	109.9	
151	C	593	1197						-5.2	120	16.7	5.24	9.90	3.06	1.42	19.98	65.5	
	I	566	1121	-4.0	220	-2	-6	+1	-5.3	117	54.2	4.88	10.98	5.76	2.61	28.13	92.3	3.1
10.5 kg	P	594	1201						-5.7	99	41.6	4.93	10.47	3.96	2.10	26.40	86.6	
157	C	523	922						-3.7	127	27.1	4.15	11.72	2.22	1.70	22.22	71.3	
	I	521	892	-3.0	216	24	36	+1	-3.6	116	29.8	4.95	11.75	2.10	2.02	26.42	84.7	0.3
10.2 kg	P	500	850						-2.9	124	21.4	4.90	9.75	2.26	2.18	21.93	70.4	
162	C	508	995						-4.3	116	22.3	4.24	13.65	2.56	0.87	19.21	64.1	
	I	516	981	0.0	200	0	12	+3	-4.0	128	20.6	5.12	12.72	2.06	1.08	20.97	66.0	-0.2
9.5 kg	P	508	956						-4.7	128	18.1	5.75	12.58	2.42	1.80	20.92	67.2	
164	C	577	1242						-4.0	125	21.0	4.48	10.70	3.22	2.30	24.43	81.5	
	I	609	1259	+5.5	280	-14	34	+1	-5.3	126	52.3	4.98	15.35	6.60	2.88	45.33	151.3	2.5
13.0 kg	P	566	1175						-5.5	123	33.8	5.32	12.35	2.91	2.91	32.50	108.5	
166	C	480	905						-4.0	150	7.6	2.90	8.43	0.25	1.10	8.74	28.3	
	I	481	895	0.0	210	4	9	+5	-4.1	145	13.2	3.27	9.03	0.37	1.43	13.55	43.5	0.6
10.0 kg	P	456	845						-4.1	148	10.5	3.46	7.82	0143	1.82	12.25	39.3	
169	C	622	1035						-2.6	123	14.1	3.58	11.17	0.77	2.71	22.19	69.0	
	I	622	1050	+2.0	260	22	20	-7	-3.0	117	36.5	4.26	14.70	4.25	3.13	39.84	126.2	2.2
12.1 kg	P	622	1048						-3.0	127	14.8	3.86	9.65	0.29	1.52	20.23	63.6	
172	C	790	1405						-6.1	112	39.8	4.94	15.32	5.16	2.18	31.67	105.6	
	I	785	1355	-2.5	285	20	10	+1	-6.2	113	33.4	5.04	13.12	2.32	2.60	26.81	89.5	-0.5
13.4 kg	P	714	1218						-6.2	124	29.0	5.27	14.00	3.30	2.64	27.99	95.7	

Table V - PREHYDRATED CONTROL

Dog. no.		Av. P.V. (ml)	Av. B.V. (ml)	B.V. incr. (%)	P_{Osm} chge (mOsm/l)	Av. R.A.P. (cmH_2O)	Av. B.P. (mmHg)	Urine flow (ml)	G.F.R. (ml/kg)/min	E.R.P.F. (ml/kg)/min	$U_{Na}+V$ (mEq)	U_k+V (mEq)	$U_{Osm}V$ (mOsm)	C_{Osm} (ml)	UF_i
142	C	665	1393			-2.7	114	36.1	4.48	13.53	1.72	2.74	20.90	68.6	
	I	681	1356	0.0	+7	-2.5	118	25.6	4.88	11.75	1.28	3.12	22.03	71.3	-.08
14.5 kg	P	696	1359			-2.2	118	30.0	5.51	13.48	2.77	4.44	32.97	90.1	
143	C	648	1342			-2.3	147	30.0	5.03	12.50	4.12	2.08	28.04	91.7	
	I	637	1301	-1.2	+5	-2.5	154	26.7	4.94	11.50	3.61	2.14	26.12	85.6	-0.2
13.8 kg	P	611	1240			-2.6	150	21.3	5.27	12.02	3.22	2.74	24.51	80.4	
147	C	508	917			-5.9	102	36.3	5.47	15.17	5.84	1.92	30.32	97.3	
	I	526	926	+1.4	-2	-5.8	118	11.4	5.32	11.88	1.17	1.24	13.35	43.1	-1.6
9.2 kg	P	530	919			-5.8	123	14.0	5.85	13.60	2.92	1.24	16.60	54.2	
150	C	460	977			-1.8	149	71.9	4.94	14.65	12.10	3.82	52.93	173.7	
	I	460	964	+0.3	+1	-2.6	153	27.8	5.17	12.93	3.95	2.52	28.02	91.9	-4.5
11.0 kg	P	459	938			-2.5	152	20.7	5.19	12.80	2.73	2.74	22.97	75.3	
154	C	535	1075			-3.4	136	77.9	4.28	13.52	11.87	2.36	44.98	144.3	
	I	545	1071	+1.3	+8	-3.0	140	16.6	3.44	7.98	0.96	1.20	14.99	47.4	-5.7
11.0 kg	P	560	1089			-2.7	140	14.7	3.39	7.82	0.80	1.54	15.51	49.2	
155	C	630	1137			-4.0	131	9.5	3.54	8.80	0.54	1.57	11.90	37.8	
	I	623	1111	-0.6	0	-4.4	127	11.3	4.35	10.37	1.12	1.96	15.76	50.3	+0.2
10.5 kg	P	656	1170			-4.4	129	14.6	4.66	11.23	1.70	2.96	19.54	62.7	
159	C	548	893			-5.3	105	17.5	4.34	10.65	1.74	1.71	19.13	61.2	
	I	536	909	+2.4	+1	-6.1	102	12.3	3.11	6.67	0.74	1.70	13.49	43.2	-0.6
10.4 kg	P	515	861			-6.0	113	8.9	2.94	5.70	0.19	1.38	11.38	36.3	
160	C	635	1522			-6.4	133	86.6	4.88	13.15	11.49	2.30	44.71	152.0	
	I	614	1579	+1.8	+3	-6.3	148	28.0	4.47	10.47	2.68	1.92	23.27	73.9	-3.9
13.5 kg	P	646	1560			-6.4	142	39.1	5.47	12.25	5.88	2.72	34.02	108.0	
168	C	480	1000			-3.5	142	12.1	5.40	16.72	0.78	1.66	17.60	54.2	
	I	499	942	-3.0	-7	-3.6	153	11.2	5.29	12.32	0.44	1.66	15.73	49.2	-0.1
10.7 kg	P	506	924			-3.1	149	14.3	5.64	12.70	1.95	3.48	20.53	67.5	
170	C	543	1038			-4.2	136	10.2	4.79	14.60	0.84	2.18	17.61	56.0	
	I	560	1051	+3.5	-1	-4.2	136	12.0	5.37	15.58	1.44	2.16	20.04	63.3	+0.2
11.2 kg	P	545	994			-4.2	122	11.5	4.69	12.32	1.10	2.00	17.81	56.5	